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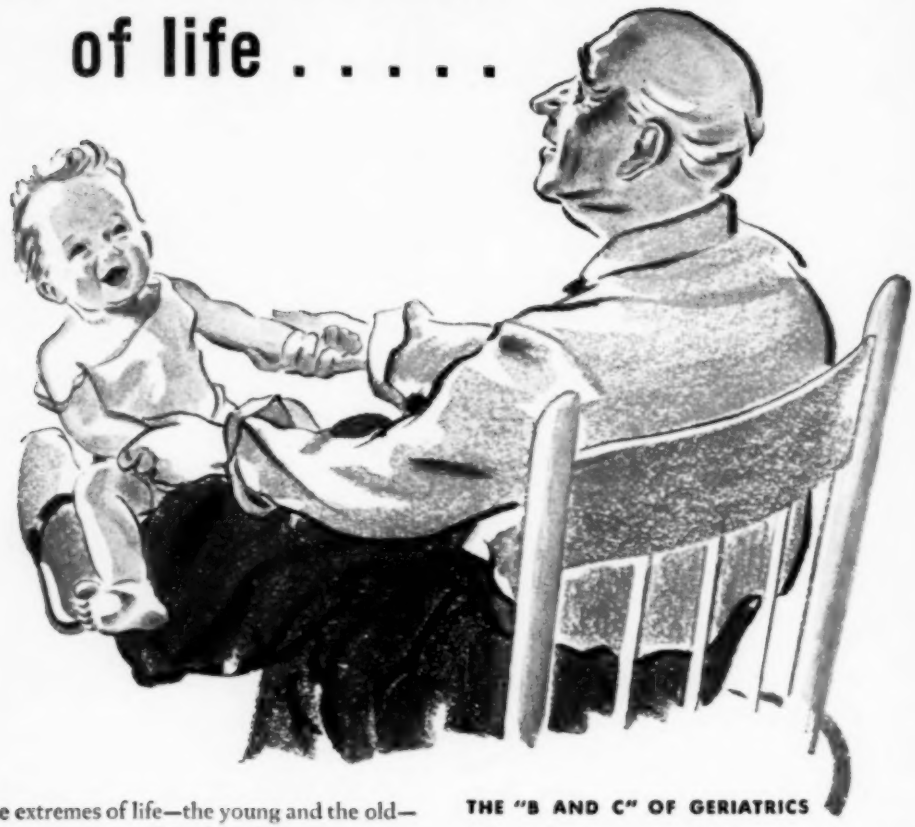

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Thiouracil in Hyperthyroidism*

By W. B. BAKER†,‡

Thiouracil may find a place in therapeutics as a remedy for hyperthyroidism. Only prolonged experience will determine its true value, its limitations, and its ultimate practical utility. The present indications are that thiouracil is a possible form of therapy for producing and maintaining a remission of thyrotoxicosis. Because the drug sometimes exhibits toxic reactions, however, it should be employed only under careful medical supervision.

WHEN over-function of the thyroid gland occurs in the animal or human body, an excessive amount of thyroid hormone is produced and released into the body. This results in a state of hyperthyroidism, or thyrotoxicosis.

The hyperthyroidism which occurs in human beings is usually manifested by an increased metabolic rate, various nervous effects, loss of weight, sometimes an increased pulse rate, and other symptoms.

In human beings, although hyperthyroidism is considered to be widely prevalent, in many cases the condition is not sufficiently serious to require hospitalization. In fact, in a number of the mild cases the disease may abate spontaneously. Therefore, it is not a reportable disease, and statistics on incidence are not easily obtainable.

When hyperthyroidism or thyrotoxicosis is sufficiently serious to require hospitalization the usual subsequent therapy, until recently, has been "subtotal surgical resection" of the hyperfunctioning gland; in other words, partial removal of the thyroid gland.

Thyroidectomy is a serious operation in hyperthyroid patients because such individuals have a high metabolic rate and, consequently, are often poor surgical risks. Although hyperthyroidism is considered to be, essentially, a medical problem the subject had been relatively dormant for years because there had not been a suitable medical treatment available. Iodine has been employed, but experience has shown that the basal metabolic rate and clinical toxicity cannot be returned to normal by iodine in the majority of cases.

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More recently, however, it has been observed that certain drugs or compounds are capable of suppressing thyroid activity by inhibiting the formation of thyroid hormone and decreasing the basal oxygen consumption in the body. In animal experiments it was noted that certain sulfonamides, as well as thiourea and its derivatives, induced a hypometabolic state when fed to rats for one to four weeks. According to accumulated evidence, the lowering of the metabolic rate results from a decrease in the production of thyroid hormone, which is presumably due to the antithyroid action of these drugs.

The earliest interest in thiourea as a medicinal hope in the treatment of hyperthyroidism actually began about the time that Kennedy and Purves (5) demonstrated that brassica seeds (i.e., rape seeds) were capable of producing thyroid enlargement in animals. It was the thiourea content of the rape seeds that was responsible for the goitrogenic effects, and later Kennedy (6) was able to produce goiters in animals by feeding allylthiourea. Other investigators who studied the effects, in rats, of many substances related to thiourea also demonstrated the goitrogenic effects of these substances (1, 2, 7). For instance, Richter and Clisby (7) obtained these effects by using phenylthiourea.

At approximately the same time as the investigations of Kennedy and Purves were reported, Mackenzie, *et al.* (3), experimented with sulfaguanidine to see whether, in killing intestinal bacteria, this drug would prevent the formation of essential nutrients by the intestinal flora. This group of investigators, because of their observations that sulfaguanidine affected the thyroid gland of rats by inducing hyperplasia and other changes, are also given early credit for much of the incentive of the later investigators who studied thiourea and its derivatives.

Although it was the Mackenzies (1) who made the original observations which led to the first clinical application of thiouracil, the real development of thiouracil therapy began with Astwood and his colleagues (2). These latter investigators confirmed the earlier work on sulfaguanidine and showed that this sulfonamide drug does not cause goiters in hypophysectomized animals. They explained that thyroid hormone is unable to be synthesized at a normal rate because of the action of sulfaguanidine or thiourea, consequently a deficiency of the hormone arises. As a result of this deficiency the anterior pituitary secretes more thyrotropic hormone and, in turn, this causes thyroid enlargement and hyperplasia.

After the Mackenzies (1) and Astwood (8) showed that thiourea and related compounds affect the thyroid gland in a striking and peculiar manner, the latter investigator tested over 100 compounds, which included a large number of thioureas as well as sulfonamides (sulfanilamide, sulfaguanidine, sulfadiazine) and other substances, and he found that thiouracil was more active than any one of them. The minimum lethal dose of thiouracil in rats was more than 100 times the dose necessary to produce a detectable thyroid effect.

A new therapeutic principle was introduced by Astwood when he reported (9) that three hyperthyroid patients treated with thiourea and thiouracil reacted favorably while the therapy was continued. Beneficial results achieved were: relief of symptoms, a decline of the basal metabolic rate to normal limits, an increase in body weight, and an increase of the body cholesterol.

From a clinical point of view, thiourea and thiouracil appeared to be the most promising compounds, and subsequently Astwood suggested the use of thiouracil in the treatment of clinical hyperthyroidism.

Interest in the thioureas, especially thiouracil, was also manifested by Rawson, *et al.* (10), after it was noted that goitrogenous patients had been made goiterous by thiocyanate therapy in hypertension. In animal experiments, these workers demonstrated that a number of thiourea compounds made the thyroid gland hyperplastic and, at the same time, a state of hypothyroidism was induced in the animals; to this extent the action of the thioureas resembled that of thiocyanate. Although this group of workers is unable to describe the mechanism of thiocyanate goitrogenesis, it is their opinion that the action of thiouracil is, probably, to block the normal iodination of protein concerned in the production of active thyroid hormones.

The actions of thiourea and thiouracil are quite similar. However, thiouracil does not give the patient an unpleasant breath, nor does it cause vomiting or conjunctivitis; thiouracil causes less gastrointestinal disturbance and it is less toxic (9, 11, 12). It is the drug of choice in the treatment of thyrotoxicosis.

The published experimental and clinical

evidence, to date, dealing with the relief of thyrotoxicosis by thiouracil therapy has aroused considerable interest and is definitely encouraging. The drug is considered by many to be valuable and potent; in fact, one investigator (13) considers it to possess life-saving properties.

Whereas only a few hundred clinical cases in which thiouracil therapy was employed have been reported, Williams (14) states that actually there have probably been more than 2000 patients treated with the drug. Evidently drug resistance to thiouracil is not manifested during therapy, since no cases have been reported.

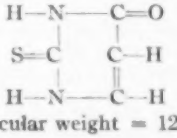
Although the ability of thiouracil to reduce the basal metabolic rate has been definitely proved, no claim for the cure of hyperthyroidism has been made up to now. Permanency of improvement and end results of thiouracil therapy have not yet been demonstrated (15).

On the other hand, it is stated that clinical remission and the normal basal metabolic rate can be maintained, apparently, as long as thiouracil therapy is employed; but if therapy is discontinued, after a few weeks a relapse usually appears within two to four weeks (14). It is thought possible that certain types of hyperthyroidism, i.e., that appearing during stress (puberty, adolescence, menopause) may yield permanent results in treating the disease, while others will not (16).

CHEMISTRY OF THIOURACIL

Thiouracil (2-thiouracil) is a heterocyclic derivative of thiourea. (Thiourea is the sulfur analogue of urea.) Thiouracil is a white crystalline powder. It is soluble in alkaline solutions, slightly soluble in water (approximately 1:2000) and insoluble in acid, ether, and ethyl alcohol; it is relatively stable either in powder form or in solution; odorless; taste, bitter.

Thiouracil, $C_4H_4N_2OS$, has the following structural formula:



Molecular weight = 128.15

ANIMAL STUDIES

Rats, guinea pigs, and tadpoles have been used experimentally as test animals in the study of the action of thiouracil.

In rats, when thiouracil was administered as a 0.1% solution in the drinking water, the drug seemed to inhibit the production of thyroid hormone and prevented the accumulation of iodine in the gland (17).

It has been shown that thiouracil in rats is much more goitrogenic than thyrotropic hormone, whereas in guinea pigs the hormone is more goitrogenic. In guinea pigs given thyrotropic hormone as well as thiouracil, the animals had much larger thyroid glands than those receiving thiouracil alone. The thyroid glands of animals receiving potassium iodide were the largest of all; therefore, it is obvious that this drug did not prevent thyroid enlargement. On the other hand, in rats, there is not much difference in the size of the thyroid gland whether the animal is receiving thiouracil alone or in conjunction with the thyrotropic hormone therapy (18).

ABSORPTION, DISTRIBUTION, AND EXCRETION

It is stated (14) that the major portion of thiouracil absorption probably occurs in the stomach and duodenum, since the drug appears in the blood in appreciable quantity very rapidly; only a small proportion of the drug has been found to be distal to the duodenum. The duodenum and jejunum can absorb the drug very rapidly. Although some thiouracil is broken down in the gastrointestinal tract, none of the drug is excreted in the stools. When incubated with the drug *in vitro*, the contents of the stomach, duodenum, and jejunum exhibit a destructive action. The gastrointestinal tract destroys approximately 15% of the usual clinical dose (0.1 to 0.2 Gm.) of the drug, and the remainder is distributed throughout essentially all body fluids and tissues. A greater concentration of thiouracil is acquired in the pituitary, thyroid, adrenals, and bone marrow than other tissues; skin and muscle contain less. Blood cells contain several times as much as plasma; the red cells possess a larger amount than the white cells. Both granulocytes and lymphocytes carry thiouracil. More of the drug is excreted when 0.2 Gm. is given once or twice a day than when 0.1 Gm. is given twice as often.

When thiouracil therapy is discontinued, the drug is not usually demonstrable in the blood or urine after three days; only a small amount is present twenty-four hours after the discontinuation

CLINICAL STUDIES

Action.—The effects of thiouracil when administered to thyrotoxic patients are (*a*) a lowering of the metabolic rate to normal levels, (*b*) a return to normal of the protein-bound iodine of the plasma, (*c*) concurrently a clinical remission of the disease. It is said to be an accepted view that the symptoms of thyrotoxicosis, which are due to excessive production of thyroid hormone, can be controlled by thiouracil administration (19). Although the primary action of the drug is centered on the inhibition of thyroid hormone production (1, 2), the exact mode is unknown.

It would appear that the site of action of thiouracil is within the gland itself, inasmuch as it has been shown that thiouracil does not destroy or neutralize thyroxine nor does thiouracil have a "paralyzing" effect upon the tissue structures which might modify the hormonal action. (Animal experiments have demonstrated that thiouracil's systemic effects could be prevented by simultaneous administration of the thyroxine.) However, there is more recent evidence (10) that does indicate an interference with thyroxine production by the follicular cells of the thyroid gland. For instance,

it has been found that extracts of the thyroid gland removed from patients with goiter, preoperatively treated with thiouracil, did not produce clinical response in myxoedematous subjects.

The mode of action of thiouracil in depressing the functional activity of the thyroid gland is still considered to be largely conjectural (21). At any rate, it is paradoxical, inasmuch as the drug induces thyroid hyperplasia with diminished iodine content of the gland and is accompanied by a decrease of the hormonal secretion.

Effect of Thiouracil on the Thyroid Gland (Hyperplasia).—A deficiency of iodine, due either to low iodine intake or to ingestion of substances which interfere with iodine metabolism, will produce a goiter. Other substances, including certain cyanides, thiocyanates, etc., are also known to be goitrogenic.

Williams (14) feels that it is strange that thiouracil, as well as the other drugs employed in the treatment of thyrotoxicosis, should be spoken of as an antithyroid drug; because, even though the drug inhibits the function of the thyroid gland, it may increase the work of the gland, as shown by the hyperplasia which is sometimes produced in the thyroid gland.

There is some difference of opinion as to what effect thiouracil has on the size of the thyroid gland. One view is that the size of the gland increases (or at least there is an absence of decrease) during thiouracil therapy, especially during the initial treatment; which is apparently due to the fact that the drug prevents the synthesis of thyroxine.

One group of investigators (22), as a result of their observations in several thyrotoxic patients, expressed the opinion that when thiouracil was used in excessive quantities, a still greater enlargement of the thyroid gland may result. The possible explanation (23) is as follows: When the production of thyroid hormone is markedly inhibited, an excessive production of thyrotropic hormone results. This in turn causes hyperplasia of the acinar cells of the thyroid and certain manifestations of malignant exophthalmos. (In such cases, it may be advisable to reduce the thiouracil dosage.)

According to Bartels (24) when thiouracil is used in the preoperative management of severe hyperthyroidism, the thyroid gland remains the same in size and consistency.

Some workers (11) point out that, although thiouracil was shown to be goitrogenous in normal animals, it is difficult to evaluate accurately the change in size of the thyroid gland because after administration of the drug the gland usually becomes much softer.

According to studies conducted by Rawson and his colleagues (20), in those patients who had had no previous treatment with iodine, and whose clinical and metabolic responses were prompt and complete, the thyroids were markedly hypertrophic and hyperplastic with practically no colloid; whereas the histology of the thyroid glands of patients who had made delayed responses to treatment with thiouracil was that of minimal to moderate hypertrophy accompanied by some degree of involution. The hyperplasia which occurs is probably due to some stimulation by the pituitary thyrotropic hormone.

It is difficult to discern changes in size and consistence of the thyroid gland during thiouracil therapy. In eleven of 72 thyrotoxic patients studied (23), an increase in the size of the gland was observed during the first few weeks of treatment; and in eight cases no change was observed within six weeks. However, a distinct decrease in the size of the gland was noted, in most of the patients, after treatment had been continued more than six weeks. In general, in those patients who received the drug for more than two weeks, the thyroid glands were distinctly firmer than normal and had a "rubbery" consistence. When desiccated thyroid (0.5 to 1.5 gr. daily) was given to nine of the 72 patients for several weeks to reduce the size of the gland, the procedure proved advantageous in most of these patients.

According to the summary observations of Williams (14), the thyroid gland generally does not change appreciably in size or consistence during the first six weeks of thiouracil therapy; in some patients the gland becomes firmer and larger; in some it gets smaller and softer, and in others there is no change.

It was also observed (23) that patients treated with thiouracil for as long as fifteen days tended to have, as determined immediately after thyroidectomy, as great a concentration of thiouracil in the glands as did those treated for longer periods of time; the values of thiouracil ranged from 5 to 39 mg. % of dried gland. It was also noted that previous iodide therapy usually had no effect on the amount of thiouracil present.

It is presumed (15) that the thyroid gland hyperplasia which occurs with thiouracil therapy is a secondary phenomenon, described as a "hyperplasia of frustration," occurring in response to the induced relative *hypo*thyroidism. However, it is stated (14) that the early fears of the goitrogenic effects of thiouracil seriously handicapping its use are no longer justified.

Length of Thiouracil Therapy.—There is a variable latent period, following the initiation of thiouracil treatment, before the metabolic rate begins to fall. Eventually clinical improvement is subjectively and objectively apparent. The fall in basal metabolic rate and amelioration of symptoms of hyperthyroidism are maintained as long as the drug is continued.

Thiouracil vs. Iodine.—Iodine and thiouracil are the only antithyroid substances which have been given much consideration clinically. Although iodine has been somewhat helpful in the treatment of certain thyrotoxic patients, it has several disadvantages. For instance, sometimes the thyrotoxicosis is refractory to iodine, either at the beginning of treatment or after prolonged iodide therapy. Also, with iodine, there is only partial improvement in many patients. The exact action of iodide in the treatment of hyperthyroidism is obscure, and there is a need for a drug with stronger antithyroid action (14).

Some thyrotoxic patients who receive iodine or iodide prior to treatment with thiouracil tend to be resistant to thiouracil therapy, especially during the first few weeks. In other patients who have been treated with iodide for six months or longer prior to the beginning of thiouracil therapy, the response in the clinical status and in the basal metabolic rate tends to be much slower than when no iodide had been given. However, when thiouracil is given concomitantly with iodide, improvement of the patient may occur although, generally, the response may be slower than when thiouracil alone is used. In those cases where the response to thiouracil is retarded because of prior iodine administration, the dosage of the thiouracil should not be increased. Generally, patients treated for several weeks with

thiouracil and without iodine usually have an extremely smooth course (14, 23).

In an attempt to make the thyroid gland more suitable for surgical removal, Bartels (24, 26) employed Lugol's solution in conjunction with thiouracil. However, he is of the opinion that further experience is indicated before proper evaluation is achieved.

In the patients observed by Rawson, *et al.* (20), the best clinical responses to thiouracil were in those rather acute cases which had had no previous treatment with iodine; whereas patients who had received iodine previous to thiouracil treatment made much slower responses to the drug. A probable explanation of the delayed action in the latter cases is that, in such patients, there probably is considerable stored hormone which, after the administration of thiouracil is started, continues to come out even though new hormone does not.

A number of differences may be noted in comparing thiouracil with iodine. Thiouracil causes a complete remission in every instance if continued long enough; whereas iodine does so in only 50% of the cases, another 30% to 40% showing partial remission and the remainder no effect at all. The speed of remission is much greater (2 to 6 times faster) with iodine but the situation is only controlled temporarily. In the case of thiouracil, the metabolism is controlled as long as the drug is continued. Iodine, however, is said to have an advantage in being less toxic (25).

There is a slower drop in the basal metabolic rate of patients who have received iodine before thiouracil administration. Many patients, especially those who are more toxic and who have had hyperthyroidism for a longer time, fail to respond sufficiently, when iodine is used preoperatively, to permit surgery without risk. In such patients, usually the thyroid gland is large, the basal metabolic rate is high, considerable weight loss indicates widespread visceral strain, and at times cardiac complications occur (24, 26).

It was considered significant that in three of nine cases, in which unfavorable results were achieved with thiouracil, iodine treatment had been given previously. Furthermore, two of the three patients had been treated with thiouracil for only three weeks, and the third patient for four weeks, then the drug had been stopped. Consequently, this relatively short treatment time in patients who had been on iodine therapy might have accounted for failure to show an effect on the pulse rate (12).

Thiouracil lowers the basal metabolism faster than Lugol's solution, therefore it is more useful than iodine in controlling hyperthyroidism. This was shown in a study of nine hyperthyroid patients by McGregor (13), who also stated that previous iodine treatment (Lugol's solution) retards the effect of thiouracil. This investigator noted a definite subjective improvement, in the patients, within a few days following thiouracil administration. He also stated that thiouracil seems to be the ideal treatment for postoperative recurrent hyperthyroidism.

Watson and Wilcox (15) point out that, whereas persistent iodine therapy is known to develop into refractoriness on the basis of what is known up to the present time, this tendency does not appear to be the case when thiouracil is employed. These investigators also call attention to the much slower response to thiouracil in patients who have been treated with iodine.

Exophthalmos.—Thiouracil has no appreciable effect on the exophthalmos ("popping out" of the eyes) which is manifested by thyrotoxic patients, according to the observations of certain investigators. For instance, in a study of 32 patients with thyrotoxicosis, it was reported (12) that treatment with thiouracil did not noticeably alter the exophthalmos; nor was this unexpected. In the majority of cases the goiter appeared to be unaffected, although in two cases an increase in the size of the goiter occurred during treatment.

In another series of cases studied (27), the exophthalmos which was present in a few of the 27 thyrotoxic patients observed was not appreciably influenced by treatment; nor did enlargement of the thyroid diminish to any extent.

An exaggeration of exophthalmos, or an increase in size of the goiter, may possibly occur if thiouracil is administered until the basal metabolic rate is below normal. It is probable that this may be avoided by administering desiccated thyroid, ½ to 1 gr. per day, beginning the administration when the basal metabolic rate has been within normal range for a week or two (16).

Means and his colleagues (22) contend that the manifestations of malignant exophthalmos may be accentuated during thiouracil therapy. Williams (14) states that exophthalmos of the nonmalignant type usually shows marked improvement or disappearance during the first few weeks of treatment with thiouracil, whereas the malignant type often increases in severity.

PREOPERATIVE USE OF THIOURACIL

In the preoperative treatment of hyperthyroid patients, the return of the basal metabolic rate to near normal levels would imply an ideal operative state with a minimum of anesthesia and postoperative reaction. Experience has shown that, in severe hyperthyroidism, iodine cannot return the basal metabolic rate and clinical toxicity to normal, whereas thiouracil will accomplish this. Therefore, thiouracil is favored by a number of clinicians as a preoperative adjunct in the severely sick patient.

THIOURACIL VS. SURGERY

It has been known for a long time that subtotal thyroidectomy in the treatment of thyrotoxicosis, while affording a measure of relief, has not been the final answer. Consequently, the discovery of thiouracil as a potentially successful medical treatment, to employ instead of surgery, has been of great interest to clinicians.

The chief advantage of treatment with thiouracil is the possibility of controlled suppression of thyroid hyperactivity and the ready reversibility of the drug. In spite of the possible necessity for permanent treatment and the possible toxic reactions of the drug, it is often preferable to subtotal thyroidectomy because of the risk of surgery and the dangers of postoperative myxedema and residual thyrotoxicosis. However, some workers (11) are of the opinion that it is still a little too early to determine whether thiouracil will prove to be a satisfactory substitute for the surgical treatment of diffuse toxic goiter, because of the relatively limited experience with the drug.

With thiouracil therapy at hand, some patients may not have to be thyroidectomized. This viewpoint was adopted after a study (13) of nine patients who were successfully treated with the drug. As a result of the study, the clinician expressed the

opinion that thiouracil would be the ideal treatment where a patient is not a good operative risk, i.e., as in severe hypertension and in patients with psychotic tendencies.

DOSAGE AND ADMINISTRATION

When Astwood (9) originally recommended the use of thiouracil in the treatment of hyperthyroidism, he suggested that the drug be administered orally in a daily dosage of 0.2 to 1.0 Gm. (average 0.5 Gm.) daily, to be lowered as the basal metabolic rate falls. In the few patients observed at that time, he noted this amount of drug effected a relief of symptoms, return to normal of the serum cholesterol and the basal metabolic rate. He observed a latent period of one to two weeks before the effects occurred, a sustained remission during treatment, and a return of the hyperthyroidism occurred when the drug was discontinued which suggests that continued administration is necessary to control hyperthyroidism.

Based on the above several cases, Astwood felt that possibly 1 Gm. of the drug daily was excessive. In one case it was apparent that 2 Gm. daily was not more effective than 1 Gm. and since a severe toxic episode (agranulocytosis) occurred in the patient, perhaps the 2 Gm. dose was the cause.

Other clinicians employed essentially the dosage advocated by Astwood. For instance, Rawson, *et al.* (20), treated patients by administering an initial dose of 0.6 Gm. per day by mouth, with a reduction to 0.4 Gm. per day after the basal metabolic rate had dropped halfway to normal.

In order to determine the correct maintenance dose for each patient, it has been suggested that the use of thiouracil be controlled by determining the patient's basal metabolic rate every ten days. The source of this suggestion (19) reports that the initial dose of thiouracil is usually 0.1 Gm. given five times a day for ten to fourteen days, after which the symptoms are greatly improved; the heart rate and basal metabolic rate fall and the body weight goes up. Then, after three weeks on the initial dosage, it is usually necessary to reduce the dose to 0.2 or 0.3 Gm. daily.

In 32 patients treated with thiouracil, it is reported (12) that the dosage was 0.2 Gm. administered five times daily, and the average duration of the initial course of treatment was twenty-nine days. Much smaller maintenance doses were required than for establishing control. For instance, the maintenance dose of one patient was only one-fifteenth of the initial dose, and yet the patient maintained weight and symptoms did not return. It is felt that the use of thiouracil should be reduced to the minimum required to maintain the weight.

Williams (14) outlines a regimen for thiouracil therapy as follows: The beginning dosage is usually 0.4 to 0.6 Gm. daily; after two weeks it is 0.3 or 0.4 Gm. daily; after a normal basal metabolic rate is obtained it is 0.2 or 0.3 Gm., and after a total of about two months of thiouracil therapy only 0.1 or 0.2 Gm. daily is given. From the point of view of ideal therapeutic effectiveness it would seem that thiouracil should be administered every three hours, day and night, but such a regimen is inconvenient and is not a necessity. In fact, a satisfactory response is obtained if no more than three doses per day are given.

OTHER POTENTIAL USES FOR THIOURACIL

The thought has been advanced that thiouracil might be beneficial in certain cardiac conditions. For instance, one report (12) mentions that the drug may be useful in very severe chronic congestive heart failure. Nussey (27) noted auricular fibrillation in four of the 27 thyrotoxic cases he observed. Two of the four cases reverted to normal rhythm spontaneously during the course of treatment with thiouracil. More recently, another investigator (28) has suggested that the therapeutic efficacy of thiouracil, in seven out of a series of 10 patients with angina pectoris, makes the drug appear as a valuable and preferable substitute for thyroidectomy. Four out of the 10 patients became entirely symptom free during the treatment.

On the other hand, Astwood (29) does not consider the use of thiouracil in cardiac patients as a practical procedure to follow. The disadvantages mentioned are the lengthy period of administration required, and the possibility of goiter stimulation or development.

Other possible uses for thiouracil which may warrant investigation include: psychoses which are partly manifested by increased thyroid action, and minor mental anxiety states (13).

PRECAUTIONS

Thiouracil is very dependable, in most cases, in controlling thyrotoxicosis. Some clinicians are of the opinion that hospitalization is unnecessary and that the majority of patients can continue with their daily work (23). Others (12, 15) feel that the drug should be given only under strict control, inasmuch as it is powerful and potentially dangerous.

TOXICITY

In Astwood's studies on rats, using more than 100 compounds, thiourea appeared to be the least toxic substance of the entire series (2). However, thiouracil was the most highly active compound tested. As stated previously in this article, thiouracil also has other advantages over thiourea. Other animal studies (30), also using rats as the test animals, appeared to demonstrate that thiouracil is relatively nontoxic, although certain toxic effects were manifested with continued large doses of the drug.

The experiences of clinicians who have observed thyrotoxic patients for toxic manifestations, during thiouracil therapy, have been varied. One clinician (14) states that toxic reactions to thiouracil have been encountered in approximately 10% of the patients, and that most of the complications have appeared in the first five weeks of treatment.

Many investigators have reported that thiouracil is well tolerated. It was reported (24) that, in observations of 11 patients, none of the cases showed any of the toxic manifestations (agranulocytosis, dermatitis, fever, and urticaria) reported by others. In another series (15) of 11 thyrotoxic patients who were treated with thiouracil, no toxic effects were noted; and periodic blood counts failed to reveal the development of anemia or leukopenia. In a different series (13) of 20 patients with thyrotoxicosis treated with thiouracil, no ill effects from the use of the drug were observed; the lowest white blood count noted in any of the patients was 4600.

Toxic reactions which have been reported with the use of thiouracil in some cases include skin rashes, urticaria, fever, headache, vomiting, diarrhea, blood changes, swelling of lower extremities, arthritis, enlargement of lymph glands, leukopenia, and agranulocytosis. The only serious toxic reactions are leukopenia and agranulocytosis and these have been encountered rarely. Agranulocytosis

is said to occur in 1% of the cases. The toxic reactions, except in the case of agranulocytosis, rapidly disappear when thiouracil is discontinued or, in some cases, when the dose of the drug is reduced.

Suggestions for Controlling and Combating Toxicity.—It has been suggested (31) that the use of thiouracil be controlled by periodic counts of the white blood cells. Mention was made of two cases, in which a marked drop of the white blood cells to 2000 per cu. mm. with 5% lymphocytes followed a short course of treatment with thiouracil. When the thiouracil was discontinued and the thyroid glands removed, the histology showed the characteristic features of hyperthyroidism and signs of involution evidenced by cuboidal lining epithelial cells and deep-staining colloids.

Himsworth (32) advises that the white cell count should not be disregarded during thiouracil therapy and that the drug cannot be continued safely if the neutrophils in the blood are falling. This clinician is of the opinion that such precautions would be valuable as a possible guide in preventing agranulocytosis in certain patients.

The need for a method of overcoming serious side reactions due to thiourea and thiouracil administration has been recognized.

Goldsmith, *et al.* (33), suggested the use of liver or folic acid in the prevention and treatment of granulocytopenia when this toxic reaction is induced by thiourea. These investigators point to experiments in rats, in which the simultaneous administration of solubilized liver almost completely prevented the neutropenic state; the granulocytopenia produced when weaning rats were given sulfonamides was corrected by liver.

Other investigators (34) expressed the hope that agranulocytosis might be prevented by administration of crude liver extract. Williams and Clute (23) used large doses of crude liver extract and pentnucleotide to combat this complication.

When a 10% solution of pyridoxine hydrochloride in physiological sodium chloride was used, in three cases of toxic agranulocytic angina, one of which followed the use of thiouracil and which developed about two months after this therapy for hyperthyroidism was instituted, the results obtained suggested that pyridoxine administered intravenously is a useful agent in this complication (35).

REFERENCES

(1) Mackenzie, C. G., and Mackenzie, J. B., *Endocrinology*, **32**, 185(1943).
(2) Astwood, E. B., *et al.*, *ibid.*, **32**, 210(1943).
(3) Mackenzie, J. B., *et al.*, *Science*, **94**, 518 (1941).
(4) Keston, A. S., *et al.*, *J. Biol. Chem.*, **152**, 241(1944).
(5) Kennedy, T. H., and Purves, H. D., *Brit. J. Exptl. Path.*, **22**, 241(1941).
(6) Kennedy, T. H., *Nature*, **150**, 223 (1942).
(7) Richter, C. P., and Clisby, K. H., *Proc. Soc. Exptl. Biol. Med.*, **48**, 684(1941); *Arch. Path.*, **33**, 46(1942).
(8) Astwood, E. B., *J. Pharmacol.*, **78**, 79(1943).
(9) Astwood, E. B., *J. Am. Med. Assoc.*, **122**, 78(1943).
(10) Rawson, R. W., *et al.*, *Ann. Internal Med.*, **19**, 829 (1943).
(11) Paschkis, K. E., *et al.*, *J. Clin. Endocrinol*, **4**, 179 (1944).
(12) Himsworth, H. P., *et al.*, *Brit. Med. J.*, **1**, 852 (1944).
(13) McGregor, J. K., *Can. Med. Assoc. J.*, **51**, 37(1944).
(14) Williams, R. H., *Arch. Internal Med.*, **74**, 479(1944).
(15) Watson, E. M., and Wilcox, L. D., *Can. Med. Assoc. J.*, **51**, 29(1944).
(16) Soskin, S., and Levine, R., *Arch. Internal Med.*, **74**, 375 (1944).
(17) Bissell, A., and Astwood, E. B., *Federation Proc.*, **3**, 5 (1944).
(18) Williams, R. H., *et al.*, *Am. J. Med. Sci.*, **207**, 701 (1944).
(19) Letters, Notes, and Answers, *Brit. Med. J.*, **1**, 313 (1944).
(20) Rawson, R. W., *et al.*, *J. Clin. Endocrinol.*, **4**, 1 (1944).
(21) Astwood, E. B., and Bissell, A., *Endocrinology*, **34**, 282(1944).
(22) Means, J. H., *et al.*, *Trans. Assoc. Am. Physicians*, **56**, 67 (1941).
(23) Williams, R. H., and Clute, H. M., *New Engl. J. Med.*, **230**, 657(1944).
(24) Bartels, E. C., *J. Am. Med. Assoc.*, **125**, 24(1944).
(25) Newman, E. V., *et al.*, *Bull. Johns Hopkins Hosp.*, **74**, 152(1944).
(26) Bartels, E. C., *Lahey Clin. Bull.*, **3**, 227(1944).
(27) Nussey, A. M., *Brit. Med. J.*, **2**, 745 (1944).
(28) Raab, W., *J. Am. Med. Assoc.*, **128**, 249(1945).
(29) Astwood, E. B., *J. Clin. Endocrinol.*, **4**, 229 (1944).
(30) Meyer, A. E., *et al.*, *Proc. Soc. Exptl. Biol. Med.*, **55**, 221(1944).
(31) Friedmann, I., *Brit. Med. J.*, **1**, 446(1944).
(32) Himsworth, H. P., *ibid.*, **2**, 829 (1944).
(33) Goldsmith, E. D., *et al.*, *J. Am. Med. Assoc.*, **125**, 847 (1944).
(34) Lozinski, E., and Simonovitch, *J. Can. Med. Assoc.* **5**, 422 (1944).
(35) Cantor, M. M., and Scott, J. W., *Science*, **100**, 545 (1944).

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Emodin
Aleudrin
Aponal
Fantan
Voluntal
2,2,2-Trichlorethanol
Sodium sulfoxylate
Anthraquinone-1,5-dicarboxylic acid
β-Furoacetyl-2,5-diethoxy aniline
Acetone dicarboxylic acid
Coronene
Di(β-butoxy-β-thiocyano ethyl) ether
dl-Homocystine
Pinite
Sparteine thiocyanate
Tetra ethyl ammonium chloride
Sabinene
1-Phenyl-3-methyl-4-oximido pyrazolone
Sodium-1,2-dihydroxybenzene-2,5-disulfonate
Tetra *n*-propyl ammonium chloride

A Chemical Assay for Ergonovine in Ergot Compared with the Biologic Assay*

By DONALD C. GROVE and BERT J. VOS, JR.

A method is described for extracting ergonovine from ergot and estimating it colorimetrically with the *p*-dimethylaminobenzaldehyde reagent. Ten samples of rye ergot assayed by this method were found to have from 16 to 32 mg. of ergonovine (expressed as the acid maleate) per 100 Gm. of ergot. Application of the method to previously assayed samples of ergot which had been fortified with known amounts of ergonovine showed a recovery of 91 per cent of the added ergonovine. Biologic assay of the same extracts as used for the colorimetric analysis gave values 8 to 20 per cent (av. = 15 per cent) lower than the colorimetric values. The discrepancy between the biologic and colorimetric assay was greater in the samples which had been ground several months prior to the assay than in those which were assayed immediately after grinding.

DURING the past several years various methods for the determination of the ergonovine (ergometrine) content of ergot have been reported; among them are those of Hampshire and Page (1), Trabucchi (2), Allport and Porter (3), Schou and Tonnesen (4), Casparis and Bullet (5), Jorgensen and Tonnesen (6), Schumacher (7), Allport and Jones (8), and Powell, *et al.* (9). The results obtained by all of these methods are undoubtedly high due to contamination of the ergonovine fraction with impurities that give a color with the *p*-dimethylaminobenzaldehyde reagent. Substances such as ergometrinine, the ergines, lysergic acids and possibly even traces of the ergotoxine group may be responsible.

With the exception of the report by Powell, *et al.* (9), no attempt was made by the above authors to show any correlation between the colorimetric estimation and biologic activity of the ergonovine fraction. If the ergonovine content of ergot is to be determined by a chemical assay instead of a biologic assay, it should be demonstrated that the results obtained by the former agree consistently with those of the latter. With this in mind the following method was devised.

EXPERIMENTAL

Method.—Ten grams of powdered ergot (nondefatted) is weighed into a 100-cc. beaker, 4 cc. of lead subacetate solution (approx. 22.5% Pb) is added, thoroughly mixed with a glass rod, and transferred to a 33 x 80-mm. paper extraction thimble, using a camel's hair brush to remove small amounts of powder remaining in the beaker. A small pledget of cotton is placed over the drug in the thimble and the thimble placed in the extraction flask (see catalog Ace Glass, Inc., No. 6725, size C). One hundred cubic centimeters of ether is poured through the thimble, the flask connected to the condenser, and the drug extracted for four hours on the steam bath.

The ether extract is transferred to a 125-cc. separator, the flask rinsed several times with small portions of ether and then with two 5-cc. portions of 1% tartaric acid solution. This gives 10 cc. of 1% tartaric acid which is used for the first shake-out of the ether and is followed by six successive 5-cc. portions of 1% tartaric acid. Generally after about the third shake-out some emulsification occurs. This can be eliminated by adding 1 cc. of alcohol after the third shake-out and 1 cc. more of alcohol after the fifth shake-out. The tartaric acid extracts are combined in a long-neck, 200-cc., round-bottom flask which is connected to the vacuum line or a water pump and the ether removed at room temperature. The solution is then transferred to a 50-cc. volumetric flask with the aid of small portions of distilled water and made to 50 cc. At this point the solution may be kept overnight in the refrigerator and the assay completed the next day.

A 5-cc. aliquot is pipetted out of the 50 cc. of tartaric acid solution of the alkaloids and made to 50 cc. with distilled water. This solution is then reserved for the colorimetric determination of total alkaloids and represents the alkaloids from 1 Gm. of drug. The 5-cc. pipette is rinsed with about 2 cc. of water and the rinsing returned to the 45 cc. of tartaric acid solution. Ammonia water (10%) is then added to this solution until the water-insoluble alkaloids have precipitated and the solution is alkaline to litmus, the mixture then being diluted to 50 cc. with distilled water.

The mixture is filtered and a 45-cc. aliquot of the filtrate is pipetted into a 125-cc. separator. One-half gram of powdered tartaric acid, 15 Gm. of NaCl, and 50 cc. of washed ether (400 cc. ether washed three times with 100-cc. portions of water to remove alcohol and filtered through cotton) are added and the mixture thoroughly shaken. (This step removes a slight amount of coloring material.) The aqueous layer is withdrawn into another 125-cc. separator and the ether washed with two 5-cc. portions of saturated NaCl solution containing 1% tartaric acid (10 cc. saturated NaCl + 0.1 Gm. tartaric acid) and the ether discarded. The washings are

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added to the aqueous solution in the second funnel, the solution is then made ammoniacal to litmus and extracted with four 50-cc. portions of washed ether. The ether extracts are combined in a 250-cc. separator and filtered through cotton into a 400-cc. beaker. The separator is rinsed with several portions of washed ether. The combined filtered ether extracts are then evaporated on a steam bath with a gentle current of air to about 20–25 cc., which is then transferred to a 125-cc. separator (the separator having been marked for a 35-cc. volume) with small portions of ether and made to 35 cc. with ether. The beaker is then rinsed with 10, 10, and 5 cc. of weak ammonia water (one drop 10% NH_3 in 200 cc. water), the rinsings being added to the separator containing the ether. The mixture is shaken fairly vigorously for two minutes (this time used for all subsequent shakings) and the aqueous layer withdrawn into a second separator containing 35 cc. U. S. P. ether and shaken. The water solution is then withdrawn into a third separator containing 35 cc. U. S. P. ether, again shaken, and finally withdrawn into a 250-cc. separator. This whole process is repeated with six successive 10-cc. portions of the weak NH_3 water. About 25–30 Gm. NaCl and 10 cc. of chloroform are added to the combined aqueous extracts. The mixture is shaken and the chloroform filtered through cotton into a 100-cc. beaker. The aqueous solution is extracted with five more 10-cc. portions of chloroform. The combined filtered chloroform extracts are then evaporated on the steam bath, with a current of air, just to dryness. (Do not allow dry material to remain on the bath for any length of time.) The residue is dissolved in 0.01% tartaric acid with the aid of a rubber policeman and made to volume with 0.01% tartaric acid, generally 50 or 100 cc. depending on the amount of ergonovine present. The solution is then run colorimetrically and biologically. The amount of ergonovine in this volume represents that obtained from 8.1 Gm. of drug.

The colorimetric determinations were carried out using 3 cc. of the alkaloidal solution plus 6 cc. of the *p*-dimethylaminobenzaldehyde reagent of Allport and Cocking (10). The colors were compared in the Brice (11) abridged photoelectric spectrophotometer using a 0.5-cm. cell and filter for 560 mμ.

Using the above procedure it was found that it was unnecessary to defat the drug previous to extraction as results obtained on the nondefatted drug were identical with those on the defatted drug. The biologic assay was done on the isolated rabbit uterus as reported by Vos (12).

Allport and Porter (3) used lead subacetate as the alkalinizing agent in their method of extraction. We have also found it more satisfactory than ammonia, MgO, etc., in that it yields less coloring matter to the ether and avoids the troublesome emulsions which otherwise occur when the ether is shaken with tartaric acid solution.

A four-hour extraction period gives reasonably complete extraction of the alkaloids as the following experiments indicate. The marc from one sample of ergot which had been subjected to the usual four-hour ether extraction was allowed to dry in air overnight. It was then mixed with 4 cc. of lead subacetate solution and re-extracted for four more hours. This second extraction increased the yield of total alkaloids by only 3.8%. A repetition of the experiment on a different sample of ergot resulted in a 2.7% increase in the total alkaloids. While such differences are minor from the standpoint of measuring total alkaloid, they would represent a serious error in the estimation of ergonovine, if the alkaloids obtained by re-extraction consisted chiefly of ergonovine. However, a determination of the ergonovine in the fraction obtained by the second four-hour ether extraction showed that this procedure would give only a 1.3% increase in the amount of ergonovine found.

Various steps have been introduced for the purpose of eliminating coloring material and other impurities; as a result the solution is practically colorless before it is shaken through the three ether washes. The purpose of the three 35-cc. ether washes is to eliminate any ergometrinine that might be present. This part of the assay was reported in a previous paper (13).

If only the colorimetric assay is desired, the combined aqueous ammonia solution after going through the three ether washes may be freed from ether, made to volume and determined colorimetrically. This solution could also be used for the biologic assay, but it contains a little ammonia and some alcohol from the ether, and in order to eliminate any effect these might have on the rabbit uterus, an extra step was introduced so that the solution to be biologically assayed contained only the dry ergonovine residue dissolved in 0.01% tartaric acid.

The method was tested for its efficiency in recovering known amounts of ergonovine in the following experiment. Three different specimens of ergot were assayed by the method: a weighed amount of ergonovine acid maleate was added to a second portion of each specimen and each of these fortified drugs was then assayed. The results obtained by colorimetric assay are shown in Table I, and indicate on the average a recovery of 91% of the added ergonovine.

TABLE I.—RECOVERY OF ADDED ERGONOVINE

Ergot Specimen	Ergonovine[a] Found by Colorimetric Method	Ergonovine[a] Added	Ergonovine[a] Found in Fortified Sample	Per cent of Added Ergonovine Recovered
A	2.01	2.00	3.75	87.0
B	2.67	2.06	4.57	92.2
C	2.34	1.93	4.16	94.3

[a] As mg. of the acid maleate per 10-Gm. sample.

The relationship between the amount of ergonovine as determined colorimetrically and that found when the same extract was tested biologically was studied on ten samples of imported rye ergot, one sample of domestic rye ergot, and one specimen of western wheat grass ergot. Replicate assays starting with the crude drug were done in the majority of cases. The results are shown in Table II.

Except for the atypical results obtained on western wheat grass ergot it will be noted that although the biologic test gives values for ergonovine which are consistently lower than those of the colorimetric method, the difference is not great. Of equal importance is the fact that the ratio of the biologic assay value to the colorimetric is quite constant, varying between only 0.80 and 0.92 with an average of 0.85. It appears likely that even more favorable results would have been obtained had freshly ground ergot been used for each assay. This variable, the length of time for which ground, undefatted ergot was permitted to stand before being assayed, was introduced into our study unconsciously, but an examination of the protocols shows that a significant negative correlation exists between the grinding-to-assay interval and the ratio of the biologic

TABLE II.—COMPARISON OF THE COLORIMETRIC AND BIOLOGIC ASSAY OF ERGONOVINE IN DIFFERENT ERGOT SAMPLES

Ergot Specimen	Total Alkaloids,[a] Mg./100 Gm.	Ergonovine[b]		
		Colorimetric, Mg./100 Gm.	Biologic Assay, Mg./100 Gm.	Biologic Assay as Per cent of Colorimetric
1	292	26.1	22.3	85.4
	290	27.7	22.6	81.5
2	282	26.4	22.5	85.4
	274	26.1	....	....
3	188	16.3	14.0	85.9
	188	15.7	14.4	91.6
4[c]	432	34.0	30.8	90.6
	...	34.4	29.9	86.9
	429	34.5	29.7	86.1
5	279	27.0	23.4	86.6
	267	26.1	23.8	91.1
6	273	18.3	14.7	80.2
	272	18.0	14.9	82.7
7[d]	398	31.7	27.1	85.6
8	241	22.5	19.1	85.1
9	254	23.7	20.9	88.0
10	243	20.7	17.2	83.0
	249	21.2	17.4	82.0
11	247	20.7	16.6	80.4
	254	19.4	16.2	83.3
12[e]	706	9.8	....	....
	691	10.0	5.1	51.0

[a] Calculated as ergotoxine ethanesulfonate.
[b] Calculated as acid maleate.
[c] A defatted ergot; results calculated on basis of defatted drug.
[d] An authentic American rye ergot.
[e] Sample of western wheat grass ergot (*Agropyron Smithii*) collected in Oklahoma.

assay result to that of the colorimetric. Thus in the four assays which were done within a month after grinding the drug, none of the ratios fell below 0.85 (av. 0.88), while on four assays done on ergot which had been permitted to stand for seven to nine months after grinding, all the ratios were less than 0.85 (av. 0.82). Such variables as the total alkaloid content of the ergot and the amount of ergonovine present had no consistent effect on the reliability of the colorimetric method.

DISCUSSION

The method described here supplies a chemical assay for the ergonovine content of crude ergot. Biologic assay gives values which are about 15 per cent lower. If these latter are assumed to give a closer estimate of the actual amount of ergonovine present, a factor such as 0.85 could be used to convert the figures obtained by the chemical method into their approximate biologic equivalents. The time-consuming biologic assay could thus be eliminated. However, in view of the slightly less satisfactory results obtained on powdered ergot which had been permitted to stand several months before assaying and the distinctly unsatisfactory results obtained on western wheat grass ergot, it appears advisable to study the possible influence of deterioration of ergot on the results by the chemical assay before dropping the biologic assay completely.

It seems not at all improbable that certain of the ergot alkaloids as they break down may pass through compounds which are physiologically inert but are still capable of giving the color reactions with *p*-dimethylaminobenzaldehyde, and whose solubility characteristics permit them to appear in the ergonovine fraction of the present separation scheme.

SUMMARY AND CONCLUSIONS

1. A method for the chemical assay of the ergonovine in ergot has been developed and compared with biologic assay.

2. Ten specimens of rye ergot assayed by this method were found to have from 16 to 32 mg. of ergonovine (expressed as the acid maleate) per 100 Gm. of ergot.

3. Biologic assay of the same extracts as used for the colorimetric analysis gave values 8 to 20 per cent (av. = 15 per cent) lower than the colorimetric values.

REFERENCES

(1) Hampshire, C. H., and Page, G. R., *Quart. J. Pharm. Pharmacol.*, **9**, 60(1936), **11**, 57(1938).
(2) Trabucchi, E., *Boll. soc. ital. biol. sper.*, **12**, 232 (1937).
(3) Allport, N. L., and Porter, G. V., *Quart. J. Pharm. Pharmacol.*, **11**, 96(1938).
(4) Schou, S. A., and Tonnesen, M., *Dansk. Tids. Farm.*, **12**, 263, 272, 279(1938); **14**, 33, 44(1940).

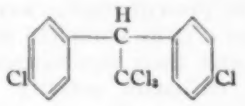
(5) Casparis, P., and Bullet, J., *Schweiz. med. Wochschr.*, **68**, 485(1938).
(6) Jorgensen, P. F., and Tonnesen, M., *Dansk. Tids., Farm.*, **14**, 134(1940).
(7) Schumacher, G., *Deut. Apoth. Ztg.*, **55**, 312(1940).
(8) Allport, N. L., and Jones, N. R., *Quart. J. Pharm. Pharmacol.*, **14**, 106(1941).
(9) Powell, C. E. Reagan, O. W., Stevens, A., and Swanson, E. E., THIS JOURNAL, **30**, 255(1941).
(10) Allport, N. L., and Cocking, T. T., *Quart. J. Pharm. Pharmacol.*, **5**, 341(1932).
(11) Brice, B. A., *Rev. Sci. Instruments*, **8**, 279(1937).
(12) Vos, B. J., Jr., THIS JOURNAL, **32**, 138(1943).
(13) Grove, D. C., *ibid.*, **30**, 260(1941).

A Synthesis of DDT*

By W. T. SUMERFORD†

A method is described for preparing a good grade of DDT in a satisfactory yield by the use of chlorosulfonic acid as a condensing agent.

THE insecticide, DDT, 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane (I), was first prepared seventy-one years ago by Zeidler



(I)

(1) who obtained the compound by reacting one mol of chloral with two mols of chlorobenzene in the presence of sulfuric acid. Although Zeidler devoted more attention to its bromine analogue, DDT has become extremely important since the discovery in 1939 of its insecticidal properties (2). Some preliminary tests indicate that the use of zinc chloride as a condensing agent gives a superior product (3), but practically all of the processes used to prepare DDT are modifications of the original method of Zeidler (1) using either chloral or chloral hydrate (4–9). While the commercial methods for manufacturing DDT are apparently good (3), it was thought worth while to look for a condensing agent, other than sulfuric acid, which might give an improved grade of DDT and possibly provide for the synthesis of some of its homologues and analogues which otherwise could not readily be prepared. Chlorosulfonic acid was found to be useful for condensing chloral and chlorobenzene if it is handled with care.

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EXPERIMENTAL

All operations were carried out under a hood. Fifteen and five-tenths grams (0.105 mol) of chloral was obtained by mixing the required amount of U. S. P. chloral hydrate with twice its weight of sulfuric acid, and distilling the chloral layer which readily separates. The chloral was dissolved in 22.5 Gm. (0.2 mol) of chlorobenzene (Eastman, Practical grade) and the solution was cooled to 5° C. under constant stirring. A temperature of from 5 to 10° C. was maintained, and to the solution, under constant stirring, were added dropwise twelve 1-cc. portions of chlorosulfonic acid (Eastman, Practical grade) at ten-minute intervals. The acid can be added safely with the use of a 5-cc. pipette. After about 8 cc. of the acid was added a solid began to separate, and within a few minutes the entire mixture became pasty. After the last portion of acid was added the stirring was continued for one hour at 10–15° C., and then for two hours at room temperature. At the end of this period the reaction mixture was mixed with chipped ice, and the crude DDT was filtered off with suction. The crude product was stirred into ten times its weight of boiling water to remove occluded acid. The product was again collected on a Büchner funnel, and washed with an equal weight of cold methanol or recrystallized once from a mixture of three parts of methanol and one part of diethyl ether. The washed product melted at 106–107° C. (uncorr.) and the recrystallized product melted at 107–108° C. (uncorr.). The yield of the purified compound was 65–70%.

REFERENCES

(1) Zeidler, O., *Ber.*, **7**, 1180(1874).
(2) British Patent 547,871.
(3) Gunther, F. A., *J. Chem. Education*, **22**, 238(1945).
(4) British Patent 547,874.
(5) Anon., *Chem. Met. Eng.*, **51**, 112 (1944).
(6) Iris, R. C., and Leyva, R. D., *Rev. inst. salubridad y enfermedades trop. (Mex.)*, **5**, 71(1944).
(7) Breckenridge, J. R., *Can. Chem. Process Ind.*, **28**, 570 (1944).
(8) Bailes, E. L., *J. Chem. Education*, **22**, 122(1945).
(9) Darling, S. F., *ibid.*, **22**, 170(1945).

Factors Influencing the Cathartic Activity of Senna in Mice*

By LLOYD W. HAZLETON and KATHLEEN D. TALBERT†

A summary of several years' experience with the mouse test for senna catharsis is presented. Among the factors considered are tolerance, deterioration, sex, weight, age, diet, temperature, and color. After long periods of feeding high dietary levels of senna, only a slight tolerance developed in males, none in females. Deterioration of fluid senna preparations may be rapid and is an important factor. Females are more sensitive than males. Within limits other factors are not significant in the method.

THE present use of large quantities of senna in the United States for cathartic preparations is evidenced by prodigious importations, as pointed out by Wiebelhaus and Lee (1). A review of the definition, habitat, commerce, and grading of the senna leaflets has been published by Grote and Woods (2).

The medicinal use of such large quantities of a crude drug poses certain problems of standardization and bioassay. Recently Geiger (3) presented a method of bioassay of senna leaves and senna preparations based on the cathartic action of senna principles in mice and by applying this method showed that low *p*H and the alcoholic content of U. S. P. Fluidextract of Senna diminish the cathartic activity of this preparation. Subsequently several reports based on this method have been published. Among these are the following: (*a*) Hazleton and Fortunato (4) reported that bile salts, under the conditions described, induce catharsis more rapidly than senna; (*b*) Wiebelhaus and Lee (1) investigated methods of producing a stable fluidextract, and also the relationship between the activity of the precipitate and the freshly prepared official fluidextract; (*c*) Hazleton and Talbert (5) observed a slow but progressive deterioration of Fluidextract of Senna, U. S. P., and that daily administration of a subcathartic dose of fluidextract of senna for several months does not significantly alter the response to the cathartic dose of the drug, indicating that no observable tolerance was developed; (*d*) Grote and Woods (2) found that of the many varieties studied only Tinnevelly senna and Alexandria senna are active in mice in doses suitable for laboratory investigation, that the T. C. D. (threshold cathartic dose—that dose which produces catharsis in approximately 50 per cent of the animals) of powdered Tinnevelly leaves in male mice is 6.3 mg., and that samples of commercial Tinnevelly senna show no loss of activity after a storage period of five years. As experience has been gained in using the method, it has become apparent that there are many factors which may influence the end point. The present studies were undertaken to obtain a more complete picture of these factors and their significance in the evaluation of cathartic activity by this method.

The drugs employed in these investigations were Fluidextract of Senna, U. S. P. (6), Syrup of Senna, U. S. P., distilled water suspension of powdered Tinnevelly Senna Leaves, U. S. P., and Infusion of Senna, U. S. P. The mouse method of bioassay described by Geiger (3) with modifications by Hazleton and Fortunato (4) was used. Each dose was administered in a volume of 0.5 cc.

EXPERIMENTAL

Chronic Feeding.—In January, 1943, fifty male and fifty female mice, approximately eight weeks old and averaging 12.5 Gm. in weight, were taken at random and segregated into cages of ten each. Thirty of each sex were given a diet of prepared dog meal to which was added 1.0% of powdered Tinnevelly senna leaves. This diet produced catharsis accompanied by loss of weight in the mice. The amount of powdered senna leaves was therefore reduced to 0.5%. The remaining forty were kept as controls under comparable housing conditions. Both groups were fed and weighed frequently and an accurate record kept on the consumption of food and on any change in weight or general physical appearance. A special feeding jar was devised so that waste of food and fecal contamination were kept at a minimum.

The average daily food consumption of the mice in the experimental and control groups is given in Table I. This average is based on monthly summaries for each cage. For female mice, 84% of the monthly averages fall within the range of 2.1 to 3.1

* Received April 11, 1945, from the George Washington University School of Pharmacy, Washington, D. C.

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TABLE I.—AVERAGE DAILY FOOD AND SENNA CONSUMPTION OF MALE AND FEMALE MICE DURING CHRONIC FEEDING EXPERIMENTS

Senna in diet	Sex	Mouse Days[a] 0.5%	Mouse Days[a] 1.0%	Food Consumed 0.5%	Food Consumed 1.0%	Food per Mouse per Day 0.5%	Food per Mouse per Day 1.0%	Senna per Mouse per Day 0.5%	Senna per Mouse per Day 1.0%
				Gm.	Gm.	Gm.	Gm.	Mg.	Mg.
Control	M	4161	422	13,202	1421	3.1	3.3	....	....
	F	5903	1903	16,767	4637	2.8	2.4	....	....
Senna diet	M	9455	1194	30,282	4337	3.2	3.6	16.0	36.0
	F	7460	1562	20,721	4065	2.7	2.5	13.5	25.0

[a] Mouse days = number of mice, adjusted with each mortality, times number of days. Figures for controls correspond to comparable periods for the mice on senna diet.

Gm., whereas only 59% of the male values are within an equal range of 2.8 to 3.8 Gm. The amount of senna consumed daily at the 0.5% level equals 2.3 cathartic doses per mouse per day in the female group and 2.5 cathartic doses in the male group, based on a cathartic dose of 6.3 mg. for male mice and 5.7 mg. for female mice. There was no noticeable toxicity or catharsis and the growth curve of the experimental group is comparable to that of the control group (Fig. 1).

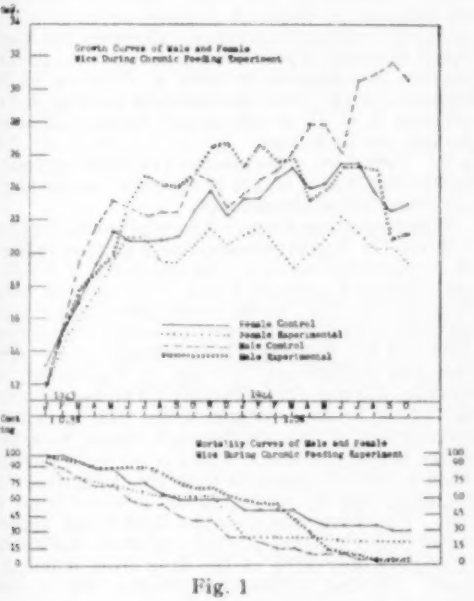
Growth Curves of Male and Female Mice During Chronic Feeding Experiment
Female Control
Female Experimental
Male Control
Male Experimental
1943
1944
Mortality Curves of Male and Female Mice During Chronic Feeding Experiment

Fig. 1

At intervals during the four hundred days of feeding on a 0.5% diet both groups were given the T. C. D. of a distilled water suspension of the powdered crude drug or a U. S. P. senna preparation, and the cathartic response was compared. At least seven days elapsed between any two tests. A comparison of all the results between the experimental female mice and the controls indicates no change in response between the two groups. However, in the male mice a comparison of results between the experimental and control animals shows that a tolerance of 25% developed in the experimental group.

At the end of the four-hundred-day test period those experimental mice remaining were again placed on a diet containing 1.0% ground senna leaves. This diet, representing 4.4 and 5.7 cathartic doses per day for female and male mice, respectively, induced an immediate softening of the stools which persisted throughout the remainder of the experimental period. Since this softening occurred in both sexes, it is apparent that the slight tolerance existing in the males was overcome and no further tolerance was developed. The persistently soft stools rendered the animals unsuitable for further testing of cathartic response to the T. C. D.

Prolonged exposure to these large amounts of senna obviously caused constant catharsis and dehydration. Since the growth and mortality curves of the experimental mice closely parallel those of the controls (Fig. 1), water balance and nutrition were not extensively disturbed. These factors, however, could account for the lower weight level of the experimental mice. During the terminal days of the experiment, because of the extreme variation in weight of the few remaining mice, the death of any one mouse caused marked variation in the growth curve of the group.

At the close of the six hundred and fifty-two experimental days those mice remaining in each group were sacrificed and a post-mortem was performed on each. There was no evidence of organic tissue damage or of abnormalities in the intestinal tract in either group.

To verify the tolerance shown in the male mice during the first experiment, a second experiment was started in July, 1944. Fifty male and fifty female mice were housed and fed under the same conditions as the first group. The results of the tests for tolerance during a period of four months so closely approximated those of the first group that they are incorporated in the summary on that group.

Oral Administration.—In July, 1943, forty-five male and forty-five female mice were segregated into cages of ten each. All of them were fed a diet of commercial mouse pellets. Thirty males and thirty females were given a daily injection (six days per week) by stomach tube of one-half the T. C. D. of fluidextract of senna. The remaining thirty were kept as controls. It was necessary to adjust the daily dose at intervals because of a change in potency of the fluidextract. This will be discussed below under "Deterioration." At intervals during a period of ten months the experimental mice and the controls were given the T. C. D. of fluidextract of senna or a distilled water suspension of the senna leaves. A comparison of all the results between the experimental mice and the controls of both the male and female groups shows that no tolerance was developed in either group following daily administration of one-half the T. C. D. of fluidextract of senna for a period of ten months. It should be noted that this routine involves daily administration of much smaller doses than does the preceding

method, but more nearly approximates the therapeutic use of the drug.

Deterioration.—It has been shown (2) that senna leaves stored for as long as five years show no deterioration when assayed for cathartic potency in mice, but that there is a progressive decrease in the cathartic potency of the fluidextract of senna. This deterioration has been the subject of considerable investigation.

As soon as the sample of fluidextract (GW-105-A) was received it was assayed for cathartic potency. At intervals thereafter the T. C. D. was determined. For female mice the potency of this sample dated June, 1943, declined 83% in seventeen months, based on the following values for the T. C. D.: June, 0.5 cc. of a 1:35 dilution; July, 1:32; November, 1:22; December, 1:16; April 1944, 1:16; August, 1:10; January, 1945, 1:6. For male mice the potency of the same sample declined 84% in seventeen months, also based on the values determined for the T. C. D. as follows: June, 0.5 cc. of a 1:25 dilution; November, 1:15; December, 1:12; April, 1944, 1:12; July, 1:10; October, 1:5; January, 1945, 1:4.

A second sample of a fluidextract (GW-113-A) dated May 27, 1944, was assayed on June 6 for cathartic potency. Its potency in female mice declined as follows: June, 0.5 cc. of a 1:32 dilution; July, 1:27; August, 1:20; December, 1:8, or 75% in seven months. In male mice the deterioration in potency was as follows: June, 0.5 cc. of a 1:23 dilution; July, 1:20; August, 1:13; October, 1:7; December, 1:6, or 74% in seven months. From this sample a U. S. P. Syrup was made in the laboratory on June 7 and assayed the following day. The T. C. D. in female mice was 1:7 and in male mice 1:5. The syrup tended to be considerably more stable than the fluidextract from which it was made, since assays conducted in December show that this same dose continued to be effective in 50% of the mice.

Less complete data on samples stored in the laboratory for periods of twenty-eight months and forty-six months have been collected. One sample dated March 6, 1941, deteriorated 67% in thirty months, 73% in thirty-four months, and 86% in forty-one months. The second sample labeled August 29, 1942, deteriorated 67% in fourteen months, 83% in eighteen months, and 93% in twenty-four months.

Two other samples dated December 1, 1942, one a syrup and the other a fluidextract, deteriorated quite rapidly, and after seventeen months neither showed cathartic potency when given undiluted in the usual volume.

Since a standard was not used simultaneously with every assay the exact rate of deterioration of each sample cannot be determined, although it is obvious that there is a gradual persistent deterioration. On those days when a standard was used, either a fresh infusion of senna or a distilled water suspension of the powdered leaf, no greater variation was observed in the response to the tested samples than to the standard.

The samples dated March 6, 1941, and August 29, 1942, were stored in amber bottles at room temperature and exposed to light, whereas the other samples were stored in transparent bottles under the same conditions. Inspection of the results does not indicate a significant difference in the rate of deterioration between the samples. Portions of several samples were stored in the refrigerator and at room temperature and the rate of deterioration studied. The most interesting of these studies was conducted on an infusion of senna made April 18, 1944. On that day 0.5 cc. of the undiluted infusion induced catharsis in 58% of the animals tested. The portion stored in the refrigerator induced catharsis in 70% of the animals tested in July, 100% in August, 90% in September, and 60% in December. The portion stored at room temperature induced catharsis in 53% of the animals tested in July, but in none of those tested in August and December. Additional information on the most favorable storage conditions and containers for preserving the activity of the cathartic principles in the senna preparations will be reported as the work progresses.

In conjunction with the study of the deterioration of senna a modified Borntrager test for potency was conducted. The results obtained do not establish a correlation between biological and chemical potency. In some samples, although there was a decline in biological potency, there was a tendency toward increase in chemical potency during a comparable period. In other samples, chemical potency decreased with decrease in biological potency. The photoelectric readings on 1 cc. of fluidextract of senna (GW-105-A) and its effective T. C. D. at approximately the same dates are as follows: 83/1:25; 122.9/1:12; 128/1:12; 133/1:4,[1] indicating an increase in chemical potency accompanied by decrease in cathartic potency. However the sample dated March 6, 1941 shows a decrease in chemical potency simultaneously with a decrease in biological potency. The photoelectric reading and the effective T. C. D. of this sample during comparable periods are as follows: 224/1:5; 185/1:5; 153/1:2.[1] Therefore, this chemical procedure appears to be unreliable as a means of chemical verification of the active principles present.

Pharmaceutical Form.—The pharmaceutical form in which the drug is presented has a marked influence on its cathartic potency. Although the T. C. D. of the powdered suspended leaf is 5.7 mg. in female mice, the T. C. D. of the senna preparations represent the following quantities of senna: fluidextract (GW-113-A), 16.0 mg. (1:32 dilution); the syrup from this fluidextract, 18.0 mg. (1:7 dilution); the infusion, 6.0 mg. (1:4 dilution). The loss or alteration of the active principles seems to be greatest in preparation of the fluidextract, since two-thirds of the potency is lost. There is no additional loss when a syrup is prepared from the fluidextract and negligible loss in preparing an infusion from the leaf. This observation on the fluidextract approximates Geiger's (3) report that 1 cc. of the fluidextract represents only 25% of the drug from which it is made. Further evidence confirming this observation has been presented by Grote and Woods (2) who reported that doses of 0.5 Gm. of Tinnevelly senna had a marked laxative effect in two human subjects, while doses of 2.0 Gm. (the U. S. P. dose) had a violent purgative action. The ratio between the U. S. P. dose of the fluidextract (2 cc.) and the therapeutic dose of the leaf (0.5 Gm.) reported by these authors is 1:4.

Time of Action.—The range in the time of action after administration of the T. C. D. of several dilutions of the fluidextract (Table II) varied considerably in the animals tested. After administration of 0.5 cc. of a 1:10 dilution, a greater percentage of females showed catharsis at the end of any given hour than the males. However, based on the ulti-

[1] Photoelectric reading on Klett-Summerson Colorimeter/effective T. C. D.

TABLE II.—TIME OF ACTION FOLLOWING ADMINISTRATION OF 0.5 CC. OF VARIOUS DILUTIONS OF FLUIDEXTRACT OF SENNA (GW-105-A) TO MALE AND FEMALE MICE

Sex	Dilution	No. Used	No. Positive	Hours 1	1¼	2	3	3½	4	5	5½	A. M.
M	1:10	42	21	0/0	5/2	...	...	61/35	...	85/42	94/47	100/50
F	1:10	43	30	0/0	...	40/28	90/62	...	...	96/67	...	100/69
M	1:12	212	118	0.8/0.4	...	8/4	41/23	...	67/32	86/40	93/51	100/55
F	1:12	202	126	2/1	...	37/23	64/40	...	68/43	79/49	...	100/62
F	1:16	190	95	2/1[a]	...	20/10	55/27	...	62/31	80/40	...	100/50

[a] At any time interval the ratio shown indicates the positive results as per cent of ultimate positives (100%)/per cent of total number of mice.

TABLE III.—CATHARTIC RESPONSE TO VARIOUS DILUTIONS OF FLUIDEXTRACT OF SENNA AND INFUSION OF SENNA IN MALE AND FEMALE MICE OF VARIOUS AGE GROUPS

Age in Weeks	Fldext. Senna GW-105-A 1:12 Male	Fldext. Senna GW-105-A 1:16 Female	Fldext. Senna GW-105-A 1:10 Male	Fldext. Senna GW-105-A 1:10 Female	Fldext. Senna GW-113-A 1:20 Male	Infusion GW-CP-1 Undiluted, Female
4	19/26[a]	...	...	5/80	...	...
5	33/51	32/47	...	...	...	...
6	35/85	32/69	...	38/50	...	...
7	21/61	...	30/37	...	...	...
8	13/76	35/23	16/56	28/60	...	...
9	20/45	11/18	5/60	...	...	...
10	...	15/46	4/75	...	...	...
11	6/50	...	...	...	11/72	...
12	...	...	...	14/75	...	...
15	54/50	55/50	...	...	...	13/84
16	...	...	...	...	19/47	11/81
17	90/45	32/43	26/47	...	...	...
19	...	...	...	...	...	15/100
21	...	...	...	...	9/77	...
27	...	...	...	...	18/83	...
28	...	...	12/25	...	...	...
33	...	...	...	...	...	14/85

[a] Number of mice used/per cent positive.

mate number of positive responses (100%) the rapidity of action in female mice following administration of a 1:12 dilution exceeds that of the male mice for the first three hours. Subsequently the rapidity of response in the two groups tends to equalize. In a dilution of 1:16 of the fluidextract, the rate of response in female mice following administration of 0.5 cc. was slower during the first four hours when compared with the results obtained after administration of a 1:12 dilution during the same period of time. At the end of five hours the rate of response was approximately equal for the two dilutions, but the total cathartic response was greater with the larger dose. At the termination of the experiments the following morning, 62% of the mice used showed catharsis following administration of a 1:12 dilution as against 50% for a 1:16 dilution. Although the majority of responses appeared in five to five and one-half hours, extending the observation period overnight resulted in an appreciable increase for those dilutions which produced approximately 50% positive response. A comparison of results in female mice shows that, during the first three hours, both the total number of responses and the rapidity of response increase with increased dosage.

Temperature.—Environmental temperature was recorded on the days assays were run. The variation in temperature covered a range of from 16.1° C. to 33.9° C. (61° to 93° F.). There was no consistent increase or decrease in response to the T. C. D. of senna and its preparations in the mice caused by this temperature variation of 17.8° C. (32° F.).

Diet.—Because of existing wartime conditions the commercial mouse pellets were not always available and it was necessary to change the diet of the mice from time to time. Other than the pellets, the mice received dog meal, dog checkers, or a mixture of pellets and checkers. At the time of change in diet, and during the period of adjustment to the new diet, there was no change in weight of the animals, and no greater variation in the response to the cathartic action of senna and its preparations than at those times when the diet consisted entirely of the commercial mouse pellets.

Age.—Groups of male and female mice of the same age, regardless of weight, were tested at weekly intervals for cathartic response to the T. C. D. of several samples of fluidextract of senna. For this purpose, a small number of animals were raised in the laboratory. The results are given in Table III. It should be noted that identical doses were given to mice of all age groups to obtain a cathartic response, indicating that the mice of the lower age groups are relatively less sensitive. In the mice which were four to eight weeks old, eight, or 61%, of the thirteen groups observed after administration of the fluidextract of senna (GW-105-A) varied more than ±10% from a 50% response. In the mice which were nine to twenty-eight weeks old, only four, or 30%, of the thirteen groups observed varied more than ±10% from a 50% response. In view of the limited number of mice used and the degree of accuracy of the test, the significance of age as an influencing factor is not well established, but it appears that a greater part of the variation in

response would be eliminated if mice at least nine weeks old were used.

Sex, Weight, and Color.—In the above discussions several observations indicate a distinct variation in response between male and female mice. Under "Chronic Feeding," it was pointed out that the average daily food consumption varied between the sexes, and that a significant tolerance existed in the male mice. Under "Time of Action" it was noted that, following administration of equal doses of the drug tested, female mice responded more rapidly and in greater numbers than the males. This latter sex variation was also noted under "Deterioration." In addition, male and female mice of equal weight and of assorted colors were given equal doses of the fluidextract and the responses observed. Most of the mice available for these studies were albino but several hundred black and brown mice were also tested. Of the 984 females used, 575 were albino, 273 black, and 136 brown. Of the 1007 males used, 616 were albino, 235 black, and 156 brown. These observations were made on one sample of the fluidextract of senna (GW-105-A). A summary of the results shows that there is no more variation in response to the T. C. D. of the fluidextract among the various color groups than there is within any one group, and no significant variation in response to the T. C. D. in various weight levels within either sex. However, there is considerable variation in response between the sexes, as pointed out above. Since this variation exists, it would seem advisable to use animals of one sex and eliminate sex difference as in influencing factor.

Route of Administration.—To evaluate the significance of the effect of the route of administration in response to the T. C. D. of senna infusion, 0.5 cc. of an undiluted infusion was administered to male and female mice by each of three routes, orally, intravenously, and intraperitoneally. The results are given in Table IV. Following intravenous injection a small number of the mice convulsed and died immediately. A few showed mild convulsions and temporary incoordination. There was no positive cathartic response. Following intraperitoneal injection, there was an occasional death and only 9% atypical positive responses. Oral administration was followed by 79% positive responses and no toxicity.

TABLE IV.—CATHARTIC RESPONSE IN MICE FOLLOWING INTRAVENOUS, INTRAPERITONEAL AND ORAL ADMINISTRATION OF 0.5 CC. OF UNDILUTED INFUSION OF SENNA

Route	No. of Mice	No. Positive	Per cent Positive	Dead	Per cent Dead
Intravenous	42	0	0	5	12
Intraperitoneal	65	6[a]	9	3	4
Oral	308	244	79	0	0

[a] Atypical responses.

SUMMARY

Feeding for four hundred days on 0.5 per cent senna diet, representing 2.3 and 2.5 cathartic doses for female and male mice, respectively, did not affect nutrition, the normal growth curve, or the normal life span in either group. While feeding on this diet the female mice showed no tolerance, and the slight tolerance developed by the male mice was immediately overcome when they were given a 1.0 per cent senna diet.

Feeding on a 1.0 per cent senna diet for 252 additional days did not affect nutrition, growth, or mortality rate.

A post-mortem on the mice remaining after six hundred and fifty-two experimental days revealed no tissue damage or abnormality in the intestinal tract.

Daily oral administration of one-half the T. C. D. of fluidextract of senna to male and female mice for ten months did not induce a tolerance to the T. C. D. in either sex.

Although the rate varies, a gradual, persistent deterioration was observed in all of the samples of fluidextract tested.

The syrup and infusion are more stable than the fluidextract. Storage in the refrigerator seems to preserve the cathartic principles.

The modified Borntrager test used proved unreliable as a means of chemical estimation of the active principles.

In using the U. S. P. method for preparing fluidextracts, 65 per cent of the active principles of senna are either lost or altered sufficiently to render them inactive.

In female mice, the total number of responses and the rapidity of response increase with increased dosage when compared with responses in male mice.

For those dilutions which produce approximately a 50 per cent response, the time of action extends beyond five and one-half hours. Extending the observation period overnight is satisfactory and assures a complete picture of total response.

No significant change in reponse within either sex results from variation in weight or color, from a temperature variation of 17.8° C. (16.1 to 33.9 °F.), or from change in diet.

In using this method, a considerable amount of variation is eliminated if mice of one sex and at least nine weeks old are used.

Injection of equal doses of senna infusion intravenously and intraperitoneally induced negligible cathartic response in mice when compared with response to the same dose following oral administration.

REFERENCES

(1) Wiebelhaus, Virgil D., and Lee, C. O., THIS JOURNAL, **32**, 165(1943).
(2) Grote, I. W., and Woods, Maribelle, *ibid.*, **33**, 208 (1944).
(3) Geiger, E., *ibid.*, **29**, 148(1940).
(4) Hazleton, L. W., and Fortunato, F., *ibid.*, **31**, 60(1942).
(5) Hazleton, L. W., and Talbert, Kathleen D., *ibid.*, **33**, 170(1944).
(6) "Pharmacopœia of the United States," Twelfth revision, Mack Printing Company, Easton, Pa., 1942.

Biological Assay of Epinephrine*

By ROBERT E. THOMPSON

Epinephrine solutions were assayed by the mammalian blood pressure method modified by application of a modern experimental design and method of interpretation. Results and reliability of individual assays were computed mathematically. Eleven epinephrine solutions of known potency were assayed on atropinized cats. The true potencies were not made known to the assayist until after computation of the results. The largest error obtained was 5.0 per cent with an average error of 1.7 per cent. Forty solutions of unknown potency were assayed on atropinized cats and dogs. The 20 assays on cats resulted in an average standard error of 2.12 per cent and the 20 assays on dogs gave an average standard error of 2.06 per cent. The largest standard error in the whole series of 51 assays was 4.7 per cent with an average of 2.2 per cent.

THE purpose of this paper is to present a modification of the mammalian blood-pressure assay of epinephrine which utilizes a modern experimental design and method of interpretation. The blood-pressure test on anesthetized, atropinized cats or dogs is the most widely accepted method for the biological assay of epinephrine (1–3). The present official method of the U. S. Pharmacopœia (3) requires that dogs only shall be used.

The assay is usually carried out by finding doses of standard and unknown which produce equal, submaximal rises in blood pressure. Chance variations in response and changing sensitivity to the drug as the experiment progresses make the data difficult to interpret. Proper interpretation of the data depends largely on the judgment and experience of the assayist.

The U. S. P. (3) requires the test animal to be standardized for uniformity of response and for sensitivity to 20 per cent difference in dosage before an assay is begun. Then the variability of the responses constituting an assay is restricted to half of the difference between the mean response to the low dose and the mean response to the 20 per cent larger dose. The U. S. P. (3) states that "evidence of potency within 5 per cent below and 5 per cent above the standard is acceptable." This evidence is, however, subject to an error of up to ±10 per cent even assuming that the same sensitivity to graded dosage prevails as during the standardization of the animal. A greater error is possible if, during the standardization of the test animal, chance variations in response indicate greater than the true sensitivity or if the sensitivity to graded dosage changes by the time the standard and unknown are compared. Uniformity of response may be incorrectly estimated or may change by the time the actual assay is run. An experienced assayist can, however, by repeated comparison of standard and unknown estimate potency to within ±5 or 6 per cent (1, 2). The number of doses necessary to obtain this accuracy is indeterminate since it depends on the sensitivity and variability of the animal being used and on the judgment and experience of the assayist.

It is evident, therefore, that the blood-pressure assay of epinephrine, together with an objective estimate of the reliability of the result, presents practically the same problems as the assays of posterior pituitary on chicken blood pressure (4–6), ergonovine on isolated rabbit uterus (7), histamine on isolated guinea-pig gut (8), etc., and the experimental designs and methods of interpretation used to improve these methods are applicable here. The experimental design and method of interpretation proposed and used by Vos (7) for the assay of ergonovine on rabbit uterus and used by Thompson (6) for the assay of posterior pituitary on chicken blood pressure is the one of choice because, as previously pointed out (6), it more effectively eliminates the effect of changing sensitivity from the experimental comparison than the experimental design proposed by Schild (8) and Smith and Vos (5).

EXPERIMENTAL

Healthy cats and dogs of both sexes were used for this study. The cats varied in weight from 1.0 to 4.0 Kg. and the dogs from 6.0 to 12.2 Kg. The animals were anesthetized with 180 mg. of sodium phenobarbital per Kg. of body weight injected in

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aqueous solution intraperitoneally. Carotid blood pressure was recorded in the usual manner using 8.5% sodium citrate as anticoagulant. No artificial respiration was used.

Intravenous injections were made directly from a hypodermic syringe by passing the needle into the exposed saphenous vein and discharging the dose at the junction of the saphenous and femoral veins.

The assay procedure evolved and used in the present experiments is as follows: A cat or dog is selected, anesthetized, set up for intravenous injection, and the carotid blood pressure recorded in the usual manner using either a membrane or mercury manometer. The animal is atropinized as directed in the U. S. P. (3) method. One cubic centimeter of U. S. P. standard epinephrine solution is diluted to 200 cc. with normal saline if a cat is the test animal or to 50 cc. if a dog is employed. The solution to be tested is diluted with the same diluent to an expected potency of 100% of the standard. Stronger or weaker solutions are used if the required volumes are not between 0.3 and 1.0 cc. for cats or between 0.5 and 1.5 cc. for dogs. A dose of the standard is found by preliminary trial which will produce a submaximal response equivalent to a rise in blood pressure of about 50 to 70 mm. of mercury (25 to 35 mm. recorded response using a mercury manometer). This dose is administered alternately with doses of the unknown which are varied so that some give responses less than the standard, others give responses approximately equivalent to the standard, and still others give responses greater than the standard. The doses of standard and unknown may be increased or decreased during the course of the assay if changing sensitivity to the drug makes it necessary in order to avoid maximal responses or recorded responses too short to be measured accurately. Each section of an assay involving a certain dose of the standard must begin and end with that dose. Likewise, in the event of an interruption, such as washing a clot from the cannula, the assay is continued but any data produced by an unknown dose not preceded and followed by a constant dose of standard are eliminated from the computation of the result.

The largest dose of unknown should be no more than twice as large as the smallest dose. The intermediate dose should be about midway between the low and high doses. It may be varied if desired to try to produce responses exactly equivalent to those of the standard, thereby producing graphic evidence of the strength of the unknown according to the principles of matching responses.

The assay should be run on a definite time schedule. The interval between doses in the present assays was five minutes. This interval is usually necessary to allow the blood pressure to return to normal and to prevent the previous dose from having an effect on the response. An interval longer than five minutes may be used if at any time it is required to allow the blood pressure to return to normal. The same interval should be used throughout a given assay.

To summarize, the doses are administred at five-minute intervals in the following order: S U_1 S U_2 S U_3 S U_2 S U_1 S, etc., where S represents the constant dose of standard and U_1, U_2, and U_3 represent the low, medium, and high doses of unknown, respectively. The number of times the doses are repeated is governed by the accuracy desired. On completion of the test the kymograph record is varnished and the responses measured to the nearest millimeter with an ordinary millimeter rule. A protocol of an assay involving 10 doses of unknown and 11 of standard is shown in Table I.

TABLE I.—PROTOCOL OF ASSAY. 10-28-43. MALE CAT 2.5 KG. 180 MG./KG. NA PHENOBARBITAL I. P.

Dose No.	Prepn.	Dose, Cc.	Recorded response, Mm.	Dose No.	Prepn.	Dose, Cc.	Recorded response, Mm.
26[a]	S	0.40	34	37	U	0.70	37
27	U	0.45	32	38	S	0.40	30
28	S	0.40	34	39	U	0.50	28
29	U	0.65	39	40	S	0.40	30
30	S	0.40	32	41	U	0.35	24
31	U	0.50	33	42	S	0.40	30
32	S	0.40	32	43	U	0.50	28
33	U	0.35	24	44	S	0.40	29
34	S	0.40	31	45	U	0.70	35
35	U	0.50	31	46	S	0.40	27
36	S	0.40	31				

[a] A previous assay involved the first 25 doses administered to this cat.

RESULTS

Calculations.—The assay data are interpreted exactly as indicated by Thompson (6) for the assay of posterior pituitary on chicken blood pressure. The responses are not expressed as logarithms as was done by Vos (7) in his assays of ergonovine on rabbit uterus. This eliminates the labor of conversion of the responses to logarithms and also makes the arithmetic easier. Either method produces practically identical results[1] because the difference in curvature of the dose-response curve over the range used is very little. Also, at least half the responses of the unknown doses approximately match the responses of the standard doses thereby reducing further any effect of curvature in the dosage-response relationship.

The estimated potency and standard error for the assay illustrated in Table I is 79.9 ± 1.4% which is in very satisfactory agreement with the true potency of 80.0% to which this unknown had been made by dilution of the standard solution.

Experimental Results.—To determine the accuracy of this method and the validity of the standard errors, 11 solutions of epinephrine of known potency were assayed on atropinized cats. The

[1] The present assays were also calculated after expressing the responses as logarithms. The use of millimeter response in the calculations for 11 assays resulted in an average error of 1.68% while the use of log response gave an average error of 1.96%. Average standard errors were 2.71 and 2.59%, respectively.

true potencies of the unknowns were not made known to the assayist until after the results were calculated. An experiment on each of the 11 solutions involved the administration of 10 to 13 doses of unknown. Each half of an experiment was designated as a separate and complete assay, resulting in 2 assays on each solution where only a relatively small number of doses were utilized for each. Results of 2 such assays on each solution are shown in Table II. This table shows the precision to be expected when 5 to 7 doses of unknown are employed in an assay. The maximum per cent error is 8.6 while the average is 2.9. By designating each experiment as a single assay the single value obtained for each solution resulted from the use of twice as many doses. The values obtained in this way are given in Table III. The maximum per cent error is 5.0 with an average of 1.7.

The term per cent standard error in Tables II and III is used to indicate comparative standard errors for the various assays. The value of the ratio, per cent error/per cent standard error, may be compared with the values in Fisher's (9) t table (with $n = N - 2$) to indicate whether the standard errors provide a valid measure of the reliability of individual assays. In Table II half of these values should be less than 0.73 to 0.77 ($P = 0.5$). Actually 12 out of 22 are less than 0.73. Nineteen values out of 20 should be less than 2.57 to 3.18 ($P = 0.05$). Twenty out of 22 are less than 2.0. Likewise, 99 values out of 100 should be less than 4.03 to 5.84 ($P = 0.01$). The highest value obtained was 3.67. These values in Table III likewise fall well within expected limits. The highest value obtained in Table III is 1.82. With 10 doses of unknown a value of 1.86 would be expected once in 10 assays, 2.31 once in 20 assays, and 3.36 once in 100 assays. The standard errors, therefore, appear to have their usual significance as a measure of the reliability of individual assays.

TABLE II.—RESULTS OF ASSAYS OF "UNKNOWN" SOLUTIONS

Assay No.	No. Unknown Doses	True Potency	Potency Found	Standard Error	% Error	% Standard Error	% Error / % Standard Error
1-*a*	7	100	105.0	8.2	5.0	7.8	0.64
1-*b*	6	100	100.5	4.0	0.5	4.0	0.13
2-*a*	6	85	82.2	0.8	3.3	0.9	3.67
2-*b*	6	85	85.6	2.2	0.7	2.5	0.28
3-*a*	5	90	82.6	5.5	8.2	6.6	1.24
3-*b*	5	90	89.5	3.6	0.6	4.0	0.15
4-*a*	5	125	134.4	14.2	7.5	10.6	0.71
4-*b*	5	125	119.4	5.7	4.5	4.8	0.94
5-*a*	5	80	77.0	2.5	4.0	3.2	1.25
5-*b*	5	80	78.2	2.8	2.2	3.6	0.61
6-*a*	5	100	102.7	2.3	2.7	2.2	1.23
6-*b*	5	100	97.6	4.6	2.4	4.7	0.51
7-*a*	5	85	83.5	0.4	1.8	0.5	3.60
7-*b*	5	85	92.3	4.4	8.6	4.8	1.79
8-*a*	5	80	81.7	0.9	2.1	1.1	1.91
8-*b*	5	80	78.3	2.7	2.1	3.4	0.62
9-*a*	5	108	107.5	1.2	0.5	1.1	0.45
9-*b*	5	108	107.1	1.8	0.8	1.7	0.47
10-*a*	5	86	87.9	2.4	2.2	2.7	0.81
10-*b*	5	86	88.5	2.7	2.9	3.1	0.93
11-*a*	5	120	120.0	4.3	0	3.6	0
11-*b*	5	120	119.9	5.2	0.1	4.3	0.02
Mean					2.9	3.7	

TABLE III.—RESULTS OF ASSAYS[a] OF "UNKNOWN" SOLUTIONS

Assay No.	No. Unknown Doses	True Potency	Potency Found	Standard Error	% Error	% Standard Error	% Error / % Standard Error
1	13	100	103.0	4.7	3.0	4.6	0.65
2	12	85	83.8	1.4	1.4	1.7	0.82
3	10	90	85.5	3.5	5.0	4.1	1.22
4	10	125	124.3	4.2	0.6	3.4	0.18
5	10	80	77.5	1.3	3.1	1.7	1.82
6	10	100	100.3	2.5	0.3	2.5	0.12
7	10	85	87.4	3.0	2.8	3.4	0.82
8	10	80	79.9	1.4	0.1	1.7	0.06
9	10	108	108.1	1.4	0.1	1.3	0.08
10	10	86	87.7	2.4	2.0	2.7	0.74
11	10	120	120.1	3.2	0.1	2.7	0.04
Mean					1.7	2.7	

[a] Same assays as in Table II but each pair (*a* and *b*) combined and calculated as one assay.

The method described has been used as an aid in the standardization of epinephrine solutions. Both dogs and cats have been used with nearly equal success. The results of the first 20 assays on cats and the first 20 on dogs are given in Table IV. Over 100 additional assays have been run with comparable results. The mean standard error for the cat assays in Table IV, 2.12, is practically identical with the mean standard error for the dog assays, 2.06, indicating that about the same accuracy was attained with either test animal. A greater number of unknown doses was given in the cat assays, however, so this series of tests indicates that on the average these dogs were somewhat less variable in response than the cats used.

usually be run in one day on the same test animal. Rarely is it necessary to discard an animal because of very rapid fluctuations in blood pressure and consequent erratic responses.

The precision of the method is greater than that of the present U. S. P. method (3). The reliability of individual assays is computed from the data of the assay itself and does not depend on a predetermined estimate of sensitivity and variability which may or may not change by the time com-

TABLE IV.—ASSAYS OF EPINEPHRINE SOLUTIONS ON ATROPINIZED CATS AND DOGS

Atropinized Cats				Atropinized Dogs			
Assay No.	No. Unknown Doses	Potency Found	% Standard Error	Assay No.	No. Unknown Doses	Potency Found	% Standard Error
1	12	107.8	3.0	21	12	102.1	3.7
2	20	95.1	3.8	22	12	107.3	2.2
3	11	135.0	2.8	23	9	87.0	1.2
4	14	72.8	3.2	24	5	92.2	2.1
5	6	103.8	1.1	25	9	104.0	1.0
6	11	104.0	3.9	26	8	97.6	1.6
7	20	85.0	2.6	27	7	78.5	2.5
8	15	96.2	1.8	28	5	77.7	1.5
9	7	91.0	1.5	29	6	99.0	1.0
10	9	89.0	2.8	30	6	90.4	1.9
11	8	98.5	0.9	31	5	115.0	1.3
12	10	92.3	2.6	32	5	99.3	2.4
13	9	88.5	1.8	33	5	86.5	4.5
14	8	90.7	3.7	34	8	97.5	0.8
15	8	110.0	1.9	35	6	103.5	1.4
16	9	113.0	1.4	36	6	101.0	1.0
17	8	99.0	1.6	37	6	106.0	3.5
18	9	100.0	0.2	38	8	105.0	3.0
19	7	99.3	0.5	39	7	101.0	2.9
20	8	101.0	1.3	40	10	93.5	1.8
Mean			2.12				2.06

DISCUSSION

Application of the experimental design and method of interpretation used by Vos (7) for the biological assay of ergonovine on rabbit uterus to the mammalian blood-pressure assay of epinephrine, as herein described, provides a simple, rapid, economical, and accurate method for the assay of this drug. Anyone familiar with manometric methods of recording blood pressure can master it. The calculations involve little more than simple arithmetic The required formulas are provided by Vos (7) and their application to the present method is identical with their application to the chicken blood-pressure assay of posterior pituitary described in detail by Thompson (6).

An assay comprising 10 doses of unknown and 11 of standard is completed in less than two hours. Three to 4 such assays can

parisons are made between standard and unknown.

The precision of the method is comparable to, if not greater, than for the usual procedure of matching or "bracketing" responses (1, 2). The results and reliability of individual assays are computed mathematically and so do not depend on the judgment and experience of the assayist for their proper interpretation.

SUMMARY

1. A method for the biological assay of epinephrine solutions is described.

2. Results and reliability of individual assays are computed mathematically.

3. The method is simple, rapid, economical, and accurate.

4. The largest error obtained in the assay of 11 solutions of "unknown" potency was

5.0 per cent with an average error of 1.7 per cent. The largest standard error obtained in 51 assays was 4.7 per cent with an average of 2.2 per cent.

REFERENCES

(1) Munch, J. C., "Bioassays," The Williams and Wilkins Co., Baltimore, 1931, p. 599.
(2) Burn, J. H., "Biological Standardization," The Oxford University Press, London, 1937, p. 114.
(3) "United States Pharmacopœia XII," Mack Printing Co., Easton, Pa., 1942, p. 266.
(4) Coon, J. M., *Arch. Internal. Pharmacodyn.*, **62**, 79 (1939).
(5) Smith, R. B., Jr., and Vos, B. J., Jr., *J. Pharmacol.*, **78**, 72(1943).
(6) Thompson, R. E., *ibid.*, **80**, 373(1944).
(7) Vos, B. J., Jr., THIS JOURNAL, **32**, 138(1943).
(8) Schild, H. O., *J. Physiol.*, **101**, 115(1942).
(9) Fisher, R. A., "Statistical Methods for Research Workers," Oliver & Boyd, London, 1938.

United States Pharmacopoeial Convention

Fifth Annual Financial Report, Covering the Period from May 1, 1944, to April 30, 1945, and Based Upon the Report of the Auditor

STATEMENT OF INCOME AND EXPENSE FOR THE YEAR ENDED APRIL 30, 1945

Income			
Sale of Pharmacopœias:			
Collections		$40,268.25	
Less: Decrease in Accounts Receivable			
April 30, 1944	$ 9,378.00		
April 30, 1945	4,868.25	4,509.75	$35,758.50
Sale of Reference Standards, A. M. A. Articles, etc.:			
Collections		$ 5,298.75	
Add: Increase in Accounts Receivable			
April 30, 1945	$ 559.50		
April 30, 1944	229.50	330.00	
		$ 5,628.75	
Less: Decrease in Collections Pending Remittance to the Treasurer			
April 30, 1944	$ 959.25		
April 30, 1945	825.61	133.64	5,495.11
Interest on Investments:			
Collections		$ 2,705.43	
Less: Decrease in Accrued Interest Receivable			
April 30, 1944	$ 811.67		
April 30, 1945	742.19	$ 69.48	
Amortization of Bond Premiums	286.06	355.54	2,349.89
Miscellaneous Income			226.85
Total Income			$43,830.35

Less:			
Expenses			
Publication and Sales Expense		$19,363.11	
Less: Increase in Inventory			
April 30, 1945	$6,630.34		
April 30, 1944	4,836.70	1,793.64	$17,569.47
Administration			3,304.97
Convention			13.46
Revision		$22,601.78	
Less: Increase in Inventory			
April 30, 1945	$2,796.18		
April 30, 1944	1,645.90	1,150.28	21,451.50
Research			7,096.52
Adjustment of Furnishings and Equipment to Depreciated Value			507.17
Depreciation on Furnishings and Equipment (Current Year)			160.25
Stationery and Supplies Inventory Adjustment			42.96
			50,146.30
Excess of Expense over Income			
For the Year Ended April 30, 1945—Exhibit A			$ 6,315.95

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS (GENERAL ACCOUNT) FOR THE YEAR ENDED APRIL 30, 1945

Cash on Deposit, April 30, 1944			$ 78,348.96
Add:			
Receipts			
Sale of Pharmacopœias		$40,268.25	
Sale of Reference Standards, A. M. A. Articles, etc.		5,298.75	
Interest on Investments		2,705.43	
Use of Pharmacopœia Text by Others		10.00	
Miscellaneous		216.85	48,499.28
			$126,848.24

Less:

Disbursements	Publications and Sales	Administration	Convention	Revision	Research	Total
Printing and Binding	$19,165.93	.....	.....	.....	.....	$19,165.93
Honoraria and Salaries	.....	$1,250.00	.....	$ 9,200.00	.....	10,450.00
Clerical	.....	825.32	$ 4.81	4,625.70	$ 179.56	5,635.39
Meetings	.....	518.33	.....	2,078.70	1,650.77	4,247.80
Supplies	.....	38.78	6.48	541.78	566.93	1,153.97
Postage and Telegrams	.....	64.82	2.17	798.07	60.05	925.11
Compensation	.....	.....	.....	.....	4,202.96	4,202.96
General	197.18	607.72	.....	5,357.53	436.25	6,598.68
	$19,363.11	$3,304.97	$13.46	$22,601.78	$7,096.52	$52,379.84

Add: Purchase—Headquarters Building	7,099.75	$ 59,479.59
Cash on Deposit, April 30, 1945—Exhibit A		$ 67,368.65

A MORE DETAILED AND SUPPLEMENTARY STATEMENT ON THE REVISION AND RESEARCH EXPENSES

REVISION—MEETINGS

Subcommittee on Scope	$1,133.24
Transportation, hotel, meals, etc., for the Revision Chairman and others, for meetings and conferences related to or required by the revision program and called by the Chairman	945.46
	$2,078.70

REVISION—SUPPLIES

Stationery	198.93
Mimeograph Supplies	180.73
Miscellaneous Office Expenses	162.12
	$ 541.78

REVISION—HONORARIA

Revision Chairman, E. Fullerton Cook	$6,416.70
Executive Assistant, Adley B. Nichols	2,783.30
	$9,200.00

REVISION—GENERAL

Reference Standards—New Standards, replenishing old standards and maintenance	$2,163.30
Booklets on the "New U. S. P. XII"	1,954.00
Inter-society Color Council	25.00
Contribution to AMERICAN PHARMACEUTICAL ASSOCIATION toward publication of Pharmaceutical Abstracts	1,000.00
Miscellaneous Expenses	215.23
	$5,357.53

RESEARCH

Subcommittee		Meetings, Supplies, Postage, Clerical, Travel and General	Technical Assistance	
1. Scope		$ 19.67	Dr. Frederick K. Bell, Asst. to Dr. Krantz	$2,374.96
5. Botany & Pharmacognosy		120.26	Kenneth Waters, Asst. to Dr. Beal	$1,800.00
6. Proximate Assays		1.20		
7. Inorganic Chemicals		91.12		
8. Organic Chemicals				
Supplies	$420.84			
Meetings	500.00			
Clerical	60.00			
Post & Tel.	25.00	1,005.84		
13. Ointments		310.96		
Anti-anemia Products Board		123.81		
Hormone Products Board		4.60		
Sterile Products Board		67.15		
Vitamin Products Board		39.39		
Digitalis Study		629.23		
Ergot Study		108.50		
Penicillin Study		399.83		
		$2,921.56		$4,174.96
				2,921.56
Total				$7,096.52

Book Reviews

Fundamentals of Pharmacology, by CLINTON H. THIENES. Paul B. Hoeber, Inc., New York, 1945. xv + 497 pp. 14 x 23 cm. Price $5.75.

Fundamentals in Pharmacology can be evaluated properly only if one keeps in mind the criteria of the entire *Medical Students Series* of which this text is one of a series under a single editorship. It is the aim of this series to publish books which give all of the fundamentals in a field omitting the nonessentials. By and large, Professor Thienes' book measures up to this standard.

This text is rather unique among pharmacology books from a viewpoint of classification. The orderly, but rather stereotyped, schemes of the reference book have been abandoned in favor of orderly progression of principles. In short, this is to be a classification of principles and not a classification of drugs. Pedagogically, something can be said for such an approach, but the reference appeal is somewhat lost.

The book opens with a chapter on strychnine. The author points out that it is a drug which everyone has heard about and its pharmacology brings a fitting introduction to many basic principles. Strychnine is followed by other central stimulants which in turn is followed by the central depressants, drugs acting on the peripheral nervous system, muscle drugs, diuretics, antiparasitic drugs, hormones and related drugs, vitamins and finally locally acting drugs and diagnostic agents.

That the plan of arrangement based on fundamental principle is not closely adhered to, is shown by the placing of a chapter on "Cellular Structure and Metabolism in Relation to Pharmacology" last. A chapter on "Pharmacy Practice and Prescription Writing" is included but is much too short.

Until a text is written in this important field for pharmacists specifically, this text has much to recommend it.—MELVIN W. GREEN.

Industrial Oil and Fat Products, by ALTON E. BAILEY. Interscience Publishers, Inc., New York, 1945. x + 735 pp. 15 x 23 cm. Price $10.00.

The basic intent of the author of this informative book is, as the title indicates, to discuss the various oils and fats of commerce, their properties and mode of manufacture. The author, who is senior chemical technologist of the Southern Regional Research Laboratory, succeeds in doing this.

The book is divided into four sections. The first section deals with a brief resumé of the nature of fats and oils. The basic chemistry and physical properties are handled in 86 pages.

Section B is concerned with the raw materials for oil and fat products. This characterization of individual fats and oils is not particularly new but is done succinctly and authoritatively.

The industrial utilization of fats and oils is considered in Section C. Pharmaceutical research workers could learn considerable from the "shortening" field about the plastic character of the fats, some of which are used in ointment medications.

The final section, Section D, deals with Unit Processes. This appeals particularly to the engineer

Although not a pharmaceutical book, this volume is packed so full of information pertinent to pharmacy that no pharmacy research library or school library should be without it. The reading of methods in other fields is always stimulating.—MELVIN W. GREEN.

The Amino Acid Composition of Protein and Foods, by RICHARD J. BLOCK and DIANA BOLLING. Charles C. Thomas Company, Springfield, Illinois, 1945. xiv + 396 pp. 16 x 26 cm. Price $6.50.

During a period when greater emphasis is placed on proteins and the individual amino acids in nutrition, it is fitting that a book dealing with analytical methods should put in its appearance. Eight of the eleven chapters in this book are devoted to a description of analytical methods for individual amino acids. The amino acids are arranged according to their chemical properties and the discussion centers about principles of separation. Chapter IX is devoted to general methods for amino acids. Chapter X consists of 14 detailed tables showing the amino acid content of the most common proteins and foodstuffs. The last chapter is a tabulation on the essential amino acids. The book is documented to the extent of 699 references.

The lack of information on the modern analytical approach by microbial assay is unfortunate. The chemical assays given are conservatively and carefully evaluated. It is apparent that much work needs to be done in this field both from a biochemical and pharmaceutical viewpoint before specificity and absolute quantitativeness can be claimed.—MELVIN W. GREEN.